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Early Development of the Amphibian Embryo under the influence of Ringer and salt solutions.

By

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(With plate X and XI.)

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Many experiments have recently been made upon the physiology of the development of various eggs in order to arrive at a better understanding of the nature of the process, and to determine, as far as possible, what forces are concerned in it.

For this purpose eggs have been subjected to the action of various drugs; they have been exposed to temperatures abnormally high or low, and have been reared under other unfavorable conditions. By a comparison of these investigations with those pursued under normal conditions several interesting and important results have been obtained, which throw considerable light on the phenomena of early development and cleavage.

But in almost every instance the experiments were performed upon the eggs of a single species, which were fertilized artificially and placed in the solution or subjected to the abnormal conditions before they had begun to segment. The results, therefore, have been somewhat limited, and it has been difficult to compare them, owing to differences in the conditions under which they were obtained. In actual life the young embryos are more apt to meet changes in their environment during later periods of growth than to find themselves at the beginning under abnormal conditions which afterward remain unchanged.

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[1]) For composition of Ringer solution see foot-note on pag. 627.

Accordingly the following experiments have been performed upon different species simultaneously, and upon the same species at different periods in its growth, with a view to determine, if possible, some relation between changes in environment and rapidity of growth, and more especially between such changes and the period of development at which they occur.

The eggs of the common spotted salamander (Amblystoma punctatum), the large wood frog (Rana temporaria), and one of the smaller wood frogs (Chorophilus triseriatus), were chosen because they seemed particularly adapted for such experiments in two essential particulars:

They are quite common in the vicinity of Baltimore, and often the three kinds can be found side by side in the same pool. The second and more important essential is that they vary greatly in the rapidity with which they develop.

The Amblystoma eggs, as determined first by CLARKE (8), and later by EYCLESHYMER (14) and others, develop very slowly.

According to EYCLESHYMER the rate of development is as follows:

Several hours intervene after fertilization before cleavage begins. For this reason these eggs are more often found in an unsegmented condition in the pools than those of the other two species. The first cleavage occupies an hour and fifty minutes or more, the second about an hour and forty minutes. From this point segmentation progresses more rapidly, but unevenly, over the two hemispheres. Gastrulation begins sixty hours after the first cleavage and the blastopore closes about eighteen hours later. The embryo escapes from the egg membranes in from eighteen to twenty-one days, and reaches the adult condition in about one hundred days.

The eggs of the large wood frog (Rana) develop more rapidly.

Only a short time intervenes between fertilization and cleavage-an hour or two. For this reason the eggs must usually be obtained very early in the morning if they are to be found unsegmented. The first cleavage occupies about an hour and the second somewhat less, while the third and all subsequent cleavages occur at much shorter intervals. Gastrulation begins toward the close of the second day, and is completed eight or ten hours later. The embryo escapes from the egg membranes during the latter half of the second week and reaches the adult condition in about ten weeks (70 days).

Being unable to find any account of the development of Chorophilus triseriatus, I was compelled to determine it for myself since this point was essential to the experiments. The development was found to be peculiar in several interesting particulars and was published in the American Naturalist for Sept. 1st 1896 (50). Briefly the results are as follows: At ordinary temperatures segmentation begins within an hour after fertilization and progresses so rapidly that gastrulation takes place during the first twelve hours, and the tadpole escapes from the egg membranes by the second or third day. There is thus a difference of $25\,{}^{0}/_{0}$ to $30\,{}^{0}/_{0}$ between the first species, while between the large and small wood frogs it is even more than this, 40 to $50\,{}^{0}/_{0}$.

In tabular form these three species present the following relative rapidity of development, the times given being the average from a large number of observations in each case:

Species	Fertilization to 1st Cleav.	Cleavage Periods			Gastrulation		External Gils	Adult Form
		1st	2nd	3nd	Begins	Ends		
Amblystoma	10 hrs.	110 mins.	100 mins.	100 mins.	60 hrs.	78 hrs.	19 days	100 days
Rana. . . .	3—5 -	75 -	60 -	50 -	42 -	50 -	14 -	70 -
Chorophilus.	1—2 -	30 -	40 -	30 -	11 -	13 -	3½ -	30 -

Methods of Manipulation.

Since the experiments were to be performed upon eggs in different stages of development no effort was made to fertilize them artificially. They were taken directly from the pools and placed in the solutions in shallow dishes under a receiver. Moistened blotting-paper was put beside them to prevent evaporation with its consequent increases in the percentage of the solution. The latter was also replaced from time to time by a fresh supply in order to secure the best results.

The eggs were kept in a well lighted room where they received the sunlight about one hour during the day. The temperature was very uniform and stood at about 70 F.

Before the eggs were placed in the solutions the stage of development was carefully determined, and as a further safeguard samples were preserved and afterward sectioned. For hardening Perenyi's weaker fluid was used followed by graded alcohols.

The author desires to acknowledge his indebtedness to Dr. E. A. Andrews of Johns Hopkins University for the suggestions which originally led to the undertaking of the experiments, and for much subsequent advice and assistance.

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Experiments with 0.6 % salt solution.

Two bunches of Amblystoma Eggs were obtained on March 7th. In one of these the eggs were unsegmented, in the other they were in the blastula stage.

Both bunches were used to verify experiments undertaken upon the same species at the suggestion of Dr. Andrews in the spring of 1895. An effort was then made to ascertain whether the results obtained by O. Hertwig (23) upon R. esculenta, and R. fusca would also hold true for Amblystoma and R. temporaria.

In Hertwig's experiments »thoroughly healthy« eggs were first fertilized and allowed to lie for an hour and a half. They were then put into six different solutions of common salt, of strengths varying from 0.5 % to 1 %. Hertwig found that the 0.6 % solution yielded the most interesting results and accordingly that strength was used in the present instance. But he was unable to carry any embryos beyond the sixth day through lack of material.

In attempting to verify his experiments it was determined to rear some of the embryos at least to the formation of the internal gills, or the true tadpole stage. The attempt proved successful and fully developed tadpoles were obtained, while Hertwig's results were verified in nearly every essential particular. There were several important differences in detail, however, and since the present investigations were carried so much further than his they furnish much that is new and interesting.

General Results.

All the eggs were removed from the gelatine, and half of each bunch were freed entirely from the surrounding membranes. Those in the blastula stage were put into a 0.4 % solution, while the unsegmented eggs were placed in a 0.6 % solution. The eggs from which the membranes were removed developed to the closing of the neural folds and then all died. Of the remainder, those in the 0.6 %

solution developed without any loss, being used up gradually in the preservation of material at regular intervals. Those in the 0.4 % solution for some reason died before escaping from the egg membranes. From a careful study of the eggs developed in the 0.6 % solution by means of surface views taken while the eggs were still alive, and by sections of the preserved material we can trace the following

Development of the Embryo. Segmentation.

Segmentation is considerably delayed and resembles very closely that of eggs subjected to a low temperature, as noted by HERTWIG (23). Sections of eggs taken during segmentation reveal the fact that the nucleus divides some little time before the protoplasm, and cells may often be found in which the nucleus has divided while the protoplasm still remains undivided. The same is true of cells at or near the centers of activity in all subsequent development. As a result karyokinetic figures are of frequent occurrence. Indeed I would venture to suggest this method of development in a 0.6 % salt solution as a useful means in karyokinetic investigation. We see clearly, therefore, that the egg is affected in its different parts unequally by the salt solution, the vegetative half being hindered more than the animal half as HERTWIG notes. There is also a corresponding inequality in each cell, the protoplasm being affected more than the nucleus, as LOEB has well shown (33).

Segmentation Cavity.

At the end of the third day the yolk cells are still very large. In section they appear as spherical masses made up of coarse granules and lying loosely in the segmentation cavity, many of them entirely free (Fig. 11). Consequently the floor of the cavity is not clearly defined as in the normal egg, but it is very irregular, and the large cells forming the yolk plug fuse toward the surface into a coarsely granular homogeneous mass. This irregularity of the yolk cells is found in subsequent stages, and makes it difficult, sometimes, to determine the exact arrangement of different parts. There are no traces of any ectoderm covering this yolk plug, the outer cells being like the inner ones save for the presence of pigment (Figs. 12 and 13).

Gastrulation.

EYCLESHYMER (14) states that »invagination always begins nearer the equator than the vegetative pole« in normal eggs, and that it starts as »an irregular broken line lying just below the equator and formed by the union of a number of cleavage furrows«. In the present instance this line is even more irregular and angular, and it begins much nearer the vegetative pole (Figs. 1 and 2). It bears the same relation to the more deeply pigmented area of the superior hemisphere as in normal development and as noted by TSUDA (39), in Rana temporaria, i. e., it starts just beneath it. The pigmented cells, however, as can be seen from Fig. 1, which represents a camera lucida drawing of the surface of a living egg, stop at some little distance from the edge of the blastopore, and the latter is wholly surrounded by large yolk cells which are entirely free from pigment. Moreover these are the largest of the yolk cells, and should be, therefore, those formed at or near the vegetative pole. CLARKE (8) states that invagination normally begins at the vegetative pole in Amblystoma, while F. HOUSSAY finds the same true for the Axolotl (26), JORDAN for the Newt (29), and TSUDA for Rana temporaria (39). The present experiments would seem to yield good testimony in the same direction.

At first the gastrula groove is much branched and follows almost exactly the boundary lines between some of these large yolk cells. But it becomes more even later on and turns upward, eventually running around the egg about one third the distance from the vegetative pole. This may be seen in Fig. 2, another surface view of a living egg farther developed. Here the furrow is crescentic and comparatively regular, but at first the convex side of the crescent, instead of the concave side, is turned toward the yolk field. I fully agree with MORGAN that the yolk exposed is somewhat more than would be enclosed by the normal blastopore, and that »any statement as to the extent of white closed over by normal embryos, based on these abnormal embryos, will give an erroneous conclusion« (39).

Formation of the Pigment.

The pigment is formed in situ in the yolk cells themselves, and can be seen as minute dark spots upon their surface. It certainly cannot be regarded as in any way of ectodermal origin for no ectoderm is formed over the yolk plug. But it does seem to be intimately associated with the centers of physiological activity as JORDAN suggests.

In normal development it does not usually appear among the yolk cells, except to darken their borders, and to color the cells along both sides of the archenteric cleft. Jordan has suggested that the relative lack of pigment in certain cells is due to a »more sluggish metabolism attendant upon less rapid cell division« (30).

We do know that where cell division is especially rapid, as in the vertebral somites and the ventricles of the brain, there is always an accumulation of pigment. The same thing exists in these Amblystoma embryos, but other conditions indicate quite conclusively that such an accumulation cannot be due merely to absolute activity, nor can the lack of it depend on sluggish division. The salt solution retards development and cell division is more sluggish than in normal embryos. This is especially true in the yolk cells. But in these embryos and more noticeably in those reared in Ringer solution, where the division is even more sluggish, nearly every yolk cell contains a large amount of pigment gathered around it's nucleus. In the external cells it appears at the surface. We have here, then, an increase in pigment combined with a decrease in activity.

The cells within the blastopore crescent are first very large and entirely free from pigment. But they soon divide and each of the resultant cells acquires a small amount of pigment near its center (fig. 2). In subsequent division the pigment is also divided and appears at the center of the resultant cells. It gradually increases in amount until at the formation of the neural folds it appears at the center of all the cells (figs. 3 and 4). At the closing of the folds (fig. 5) the pigment spots cover a large portion of the cell, and still later in development (fig. 6) they have increased sufficiently to color the whole surface of the yolk nearly as dark as the surrounding ectoderm. The cells themselves, however, remain very large compared with the ectoderm cells. There seems to be, therefore, a well defined growth of pigment in situ, and in all subsequent development the surface of the yolk is approximately the same color as the ectoderm, but traces of the peculiar mottled appearance remain for some time (fig. 6). In the normal embryo such yolk cells are not very active and they are still less so here. But there is, in all probability, a greater difference in the relative activities of nucleus and protoplasm in these eggs, and hence, compared with its surroundings, the nucleus may be considered as quite active. It is probably this relative activity of the nucleus, rather than any absolute activity, which it certainly does not possess, that accounts for the accumulation of pigment around it.

The irregularity of the border between the pigmented and the light area is very noticeable during gastrulation. This is well shown in fig. 1. At some points the pigment extends far up toward the blastopore crescent in loose irregular streaks and blotches, while elsewhere it has been advanced but little beyond the equator. The cells in the dark blotches are of the same size as those in the light areas at the same level, and are much larger than the ectodermal cells. This irregularity cannot be explained, therefore, by a difference in the down growth of the pigmented cells of the upper hemisphere. It persists even after the formation of the blastopore ring and must be due to the inequality of the yolk cells themselves, and the consequent difference in the influence of the salt upon them.

A study of sections shows that no ectoderm is formed over the yolk plug, but that this darkening is due to the formation of pigment in situ (figs. 12 and 13).

Tsuda (39) found in Rana temporaria that »the growth of pigment does not correspond in any way to the growth of new ectoderm cells, but . . . . varies in various lots of eggs of the same stage procured at different times«. That is, eggs from different females vary considerably in the amount of pigment they contain at any given stage in development.

But she further states that »all the eggs of the same stage from one cluster are alike in quantity of pigment«. In these eggs not even this was true, but different eggs from the same cluster varied considerably in the amount and distribution of pigment.

Tsuda concludes that the amount of pigment varies with the conditions previous to the beginning of segmentation. It would seem fair to add that conditions may also arise during segmentation which will modify the pigment considerably: or even cause it to appear abundantly in places where it is not usually found at all.

The Fate of the Blastopore.

Concerning this much discussed question the embryos developed in salt solution present many interesting details.

In the first place the real abnormality in such development lies in it's retardation. These Amblystoma eggs produced in twenty days embryos which in outward appearance were exactly like others reared normally in a little more than half the time (fig. 8). And a study of sections did not reveal any essential difference.

This retardation renders it possible to follow more easily the exact method of development, and we can determine the following facts:

1. There is no closing of the blastopore previous to the formation of the (temporary) anus as in normal development, but the yolk plug remains exposed until after the external gills and the heart have been formed. By this time the primitive gut is well developed and it's different parts are performing their individual functions. Hence, in these embryos the formation of an anus capable of functioning as such is not at all dependent upon a previous closure of the blastopore.

2. The anus certainly occupies the anterior portion of the blastopore so long as the yolk plug remains exposed. This seems at first sight a direct contradiction of the results obtained by Morgan, Clarke and others, but it will be found upon further examination that it really supports their statements. Let it be remembered that the retardation is more effective upon the passive yolk cells than upon the active pigment cells. Hence we may expect to find any process in normal development intimately connected with these yolk cells very much delayed. It might even be possible for one stage in the process to occupy a considerable period of time, and that is just what occurs in the present instance.

In the normal development of Amblystoma, the archenteron forms a cavity which is large and deep anteriorly, but posteriorly narrows from before backward as it passes down to the anus, while it remains wide from side to side. This was first pointed out by Morgan and has been amply confirmed during the present investigations. It is well shown in fig. 21, which represents a longitudinal median section of a normal embryo just after the closing of the blastopore. We see that in front the cavity extends down almost to the ventral surface of the embryo, while posteriorly it is pushed upward to the dorsal surface. The whole ventral portion in this posterior region is occupied by the mass of yolk cells which gradually withdraw into the blastopore as the latter closes. This closure of the blastopore, as recently determined by Eycleshymer and Morgan for Amblystoma, and by Jordan for the Newt takes place as follows: There is first a convergence of cells from all parts of the margin at a uniform rate. This process is gradually modified by the more rapid movement of cells on either side, which press in toward the mid-line until the blastopore becomes a longitudinal slit. Soon they meet along the middle of the slit and fuse, leaving a small circular orifice at either end. The anterior of these openings is the neuropore, the posterior becomes the permanent anus. In embryos developed in salt solution there is at first a similar uniform convergence of cells from all parts of the blastopore margin, but this is quickly stopped. There is no rapid growth of the cells at the sides, and consequently no elongation of the blastopore. This cannot be explained by the influence of the salt solution, for if that had any effect at all, it would increase the difference between the activity of the cells at the sides and those at the ends of the blastopore i. e. it would retard the more active side cells less than the comparatively sluggish cells at the ends.

There is also positive evidence in later development that the side cells retain their activity. We are compelled, therefore, to look elsewhere for our explanation. If we examine a median longitudinal section of one of these salt embryos at this period we find that the archenteron is of about the same depth throughout its entire length (fig. 18). The cells of the yolk plug have not withdrawn from the blastopore, and hence do not fill up the posterior part of the archenteron.

The dorsal lip of the blastopore is larger, thicker, and rounded inward more than normally. This is due to a combination of circumstances.

In the first place the lip is hindered in its downward growth by the non-withdrawal of the yolk plug. As it elongates, therefore, it would tend to enlarge at the end, to round inward, and probably to lift up the dorsal surface of the embryo somewhat.

A second reason is found in the fact just mentioned, that the sides of the blastopore border cannot move inward to meet along the mid-line. That they tend to do so normally shows that they must possess considerable activity. In the present instance some other mode of exercise must be found for this activity. It is found in a migration of cells from the lateral borders toward the dorsal mid-line, and a consequent accumulation of material at that point. This contributes to the enlargement of the dorsal lip.

3. That there is such a migration of cells, and that the closing of the blastopore is here due not to a backward extension of all the material of the dorsal lip over the yolk, but only of its central portion, as Morgan has suggested for the development of Rana, is clearly shown from the following considerations.

When first formed the medullary folds extend around the blastopore on either side nearly to it's posterior end. As they gradually fuse beginning at the anterior end of the embryo, it is evident that when the fusion reaches the blastopore it must stop there unless some such migration takes place. The folds along the sides of the blastopore cannot come together over the yolk plug.

The fusion does not stop, however, for the folds along the sides of the blastopore disappear. Furthermore the dorsal lip of the blastopore, in the median line only, begins to grow backward over the yolk plug as seen in figs. 6 and 19. Such a growth could only take place by »new tissue coming up to the middle line from the sides, and placing itself with or behind the cells already present in the dorsal lip«.

Hertwig says that in normal development the blastopore border, with the exception of the small portion which becomes the anus, thus serves for the origin of the dorsal organs, but that the greater portion of the border remained unused in his salt embryos (Rana esculenta), and simply surrounded the yolk fields.

It is interesting to note that in these Amblystoma embryos whose development was carried so much farther than that of his Ranas, we find a similar condition at first, but later on there is good evidence that the greater portion of the border does take part in the formation of the dorsal organs.

The fact that this backward growth is compelled by the presence of the yolk plug to take place horizontally instead of vertically, and the fact that the posterior lip of the blastopore remains free from yolk cells leaves the opening of the blastopore, the anus, where it was before, at the anterior end of the blastopore under the dorsal lip (fig. 12). Attention is called to the fact that during all this time the blastopore occupies the ventral portion of the posterior end of the embryo, and that the anus opens posteriorly, not ventrally.

The backward extension of the dorsal lip of the blastopore sometimes results in the production of abnormalities, owing to the fact that it remains free from the yolk.

One of the most common of these is seen in fig. 10. Here instead of growing downward over the yolk plug as is usually the case, the tail of the embryo has turned upward and grown directly away from it. This is much more common in Rana than in Amblystoma and is probably due to the fact that the cells on the ventral surface of the tail increase more rapidly than the others.

4. The blastopore retains its position at the posterior end of the embryo for several days; the yolk cells then begin to withdraw very slowly. At the same time the dorsal lip of the blastopore grows down vertically over the yolk cells, while the archenteric cavity becomes narrowed by the withdrawal of the yolk cells into its posterior ventral portion (fig. 20). This process continues slowly for five or six days, the blastopore growing steadily smaller, until finally, between the 17th and 20th days the dorsal lip has reached the ventral lip (fig. 14). The anus now occupies what was originally the posterior portion of the blastopore and opens ventrally instead of posteriorly. The embryo has become normal in its outward appearance.

During this growth the anus has changed its position gradually, and has occupied successively every portion of the dorsoventral diameter of the blastopore.

This gradual closure of the blastopore can be well seen in figs. 17 to 20, which are a series of longitudinal median sections taken from embryos in different stages of development. Figs. 12 to 14 show the enlarged posterior ends of another similar series.

5. We are fully conscious that we are dealing here with abnormal conditions of development, and that the greatest care must always be exercised in reasoning from the abnormal to the normal. But from the fact that the abnormality in the present instance is chiefly a retardation, from which a normal embryo is ultimately developed, it would seem as if we might infer that in the normal closing of the blastopore the withdrawal of the yolk cells is a more important factor than has been hitherto supposed.

They are usually regarded as inert and insignificant factors in development, remaining passive while the active pigment cells grow down over them and push them in. From a study of these embryos, however, it would seem that they are really the active agents of their own withdrawal, and when in any way their vitality is impaired or hindered, the pigment cells have to wait for them to act before they can accomplish their own work. In fact the whole closure of the blastopore, in these embryos at least, seems hinged on the withdrawal of the yolk cells. It is possible that the difference in the modes of closure of the blastopore noted by different authors may depend quite as much upon variations in the activity and mode of withdrawal of the yolk cells as upon individual peculiarities of the pigment cells.

6. It will be readily seen that a separate neuropore cannot be formed in these embryos. Ordinarily when the sides of the blastopore meet and fuse at the mid-line two openings are left, the anterior of which is the neuropore.

Here, however, as the blastopore gradually closes, the anus always occupies its extreme anterior end and there can be no opening in front of it. In the development of the Newt JORDAN shows »that the relative position of neuropore and anus cannot be constant«, but that the latter »may lie in almost any portion of the original mid-line through the blastopore« (29). Several figures are given showing different distances between anus and neuropore, the two being usually quite close together. All that separates them is a varying length of the fused sides of the blastopore. In these embryos the sides of the blastopore do not fuse and hence there is nothing to separate neuropore and anus and the two coincide in one common opening.

The neuropore is ordinarily quite transitory and even in the present slow development cannot be regarded as sharing this opening very long.

Is this fusion of neuropore and anus at the anterior border of the blastopore of any significance in determining »whether this anterior portion of the blastopore primitively opened freely, and has been only secondarily caught in as the neurenteric canal by the precocious closing of the medullary folds?« (29) It is certainly quite suggestive.

Experiments with Ringer solution[1].

A second lot of eggs was obtained on March 20th from pools covered with ice a quarter of an inch thick. This lot included all three kinds, Rana, Chorophilus, and Amblystoma. Two large bunches of Rana eggs were still unsegmented while the remainder were in the gastrula stage. The Chorophilus and Amblystoma eggs were all in the blastula stage.

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[1] The Composition of RINGER Solution is:

Normal salt solution (0.75%) . . . . 100 cc.
Potassium Chloride solution (1%) . . . . . . 3 -
Calcium Phosphate added to saturation.

Samples of each kind of eggs were placed in four different solutions, viz.

1. Ringer solution undiluted.
2. - - diluted one third with water.
3. - - - - half.
4. - - - two thirds.

The results obtained from the eggs in advanced stages of development can be considered more appropriately later, and we will concern ourselves here with the experiments upon unsegmented eggs (Rana) only.

Most of the eggs were left in the jelly as they were found except that the bunches were divided once or twice to reduce the bulk. Some of them, however, were removed from the egg membranes and placed in the solution entirely free. These developed as far as the closing of the neural folds and then all quickly died. By comparing this result with that obtained in salt solution, it seems reasonable to suppose that the closing of the neural folds represents a critical period in the development of these embryos.

Such a conclusion is strengthened by the following facts. In every bunch of eggs there is considerable difference in the rate of development. Some eggs reach the stage just mentioned sooner than others. These were usually the first to die, while those that developed more slowly lived until they reached the same stage. Thus the deaths were not simultaneous, but they did all take place at about the same development period.

Furthermore the very thing occurred which would be naturally expected-viz. a few eggs produced embryos more vigorous than the rest, and these developed for two or three days beyond the closing of the folds, and then gradually weakened and died. In every instance there was no more than the average loss previous to this stage. There must be something, therefore, nearly simultaneous with the closing of the neural folds, which renders this particular period fatal to embryos deprived of the protection afforded by the egg membranes. This something appears to be the:

Ciliation of the Larva.

This was first recognized by Clarke in '80, but has been doubted or entirely overlooked by others. Very recently (Feb. '96) Assheton (2) has worked out the ciliation of Rana temporaria, and of the Newt, Triton cristatus. He finds that the first indications of cilia in both species occur shortly before, or just at the time of the closing of the neural folds. The cilia are developed first upon the lateral expansions of the neural plates, and along its edges, and produce a current whose direction is from before backwards. Later a similar current is produced by cilia upon the ventral surface. The ciliation spreads rapidly until the whole embryo is covered, but the currents differ much in intensity, and are specially developed in certain regions.

ASSHETON concludes that there can be very little doubt that the purpose of this ciliation is respiratory. Such a conclusion is of special significance when taken in connection with the facts observed in the present experiments. In normal embryos the movement of the cilia produces not only a current in the surrounding medium, but also a horizontal rotary motion of the embryo itself within the inner egg membranes. This motion is increased by both salt and RINGER solutions and becomes so persistent that patient watching fails to catch the embryo at rest long enough to sketch it with a camera lucida. In order to obtain this it must first be killed. The cilia are so strong in some instances as to produce the same motion in embryos lying free upon the bottom of the dish, and these, so far as could be determined, were the ones which lived beyond the closing of the neural folds. Ordinarily, however, the greater amount of friction to be overcome against the bottom of the dish rendered such motion impossible. As can be seen from figs. 15 and 16 the chief areas of ciliation are the head, the neck, and a broad band extending obliquely across the sides of the body. When the embryo is freed from the membranes it's weight causes it to rest upon one side on the bottom of the dish, and consequently the cilia upon that side are rendered practically useless. In it's normal position within the egg membranes the fluid which surrounds it is denser than water, and hence more nearly of the same specific gravity as the embryo itself, and the latter has no difficulty in keeping free from the membrane. Furthermore the jelly in which the eggs are enveloped is comparatively thick and firm during earlier development, but gradually softens, and finally goes to pieces and allows the larvae to escape. There would seem to be established in this way a delicate adjustment of osmosis, whereby the larva is carried safely beyond the ciliated period and initiated gradually into the less dense medium. The rotary motion produced by the cilia, therefore, is probably of considerable importance, and its failure under adverse circumstances results fatally.

The first sign of ciliation in these Amblystoma embryos consists of a backward movement along the neural folds on the posterior surface, just as in Rana. The two figures given in figs. 15 and 16 are camera lucida drawings of normal embryos showing the currents generated by the cilia after the neural folds have closed (fig. 15), and again twelve hours later (fig. 16). The cilia are confined chiefly to the head, neck, and side of the body. Their motion can be plainly seen under a good handlens, and the currents are easily made out with the aid of a little chalk dust or other light colored insoluble powder sprinkled in the water. In the earlier stage (fig. 15) the strongest current starts from the dorsal surface just behind the head and sweeps backward and downward around the side of the neck to the ventral surface just outside the heart.

It thus covers exactly the position occupied in later development by the gills. Another current starts from the anterior extremity of the head and moves backward along the ventral surface until it joins the first near the heart. The two then divide and pass backward in a broad band along either side of the body to the posterior extremity, broadening gradually as they go. A third current seems to be a portion of the second, which separates from it on either side just below the eye and circles around the latter.

In the later stage (fig. 16), the current which passes across the position of the future gills continues to be the strongest. But now it unites with it's fellow from the opposite side, and the two, joined by the second current which maintains it's former position, proceed backward along the ventral surface. The whole side of the body is covered by a very broad and comparatively feeble current which takes its origin at the dorsal surface just behind the neck and runs backward along the side of the body, bending toward the ventral surface just before it reaches the posterior extremity.

The ciliation of these Amblystoma embryos thus resembles very closely that given by Assheton for Rana and the Newt, Triton cristatus, especially in the strong current passing over the position of the future gills. This fact serves to emphasize still further his conclusion that its function is respiratory.

With reference to the currents about the stomodeum I was unable to determine anything definite save that such currents do exist.

Comparative Influence of Solutions upon Eggs and Embryos in Different Stages of Development.

It remains for us to determine, if possible, the relation of the influence of these solutions to the rapidity of development on the one hand, and on the other to the period of development at which the embryos are first subjected to that influence.

In order to ascertain these points the following experiments were instituted.

Care was taken to determine exactly the condition of the egg or embryo before it was placed in the solution. For this purpose in all doubtful cases samples were preserved, and the final decision was made only after sectioning.

The experiments include all three species of amphibians, and many different stages in the development of each. So far as these species are concerned, therefore, the conclusions are trustworthy.

Experiment 1. A single bunch of Rana eggs was obtained on March 23rd which, when examined, proved to have the third furrow just developed.

One hundred of these were cut out of the egg membranes and placed free in a 0.6 % salt solution, with as many more left within the membranes. The remainder of the eggs were allowed to develop normally for 24 hours, when two hundred more were placed in the salt solution under the same conditions. The rest again developed normally for 24 hours and were then all put in the salt solution. All eggs placed in the solution free from the membranes developed to the closing of the neural folds with a loss of less than 10 %, but then, or within 24 hours after, they all died. Those which had been left for 48 hours had nearly reached this stage before being placed in the solution, and so lived but a few hours longer. The first lot stopped segmenting altogether for a short time when placed in the solution, and then began again. The second lot seemed to stop for a longer period than the first, and the third lot longer than the second. The actual time was not taken, so that only a rough comparison could be made, but enough was observed to suggest that the effect of the solution was increased with the age of the embryo.

This suggestion was the only outcome of the experiments, for these embryos all died on or before March 30th.

Experiment 2. Some Rana eggs in the 4 cell stage were removed from the jelly, but left within the membranes, and placed in Ringer solution on March 27th.

They remained dormant for about two hours (this time is the average for all the eggs, of course there were many variations due to individual differences). They then gradually recovered and developed in all respects like those reared by Hertwig and others in salt solution.

In later development these eggs showed the appearance and gradual growth of the cilia over the ectoderm very finely. It was possible to verify upon them the ciliated tracts and currents given by Assheton for the same species. For twelve days there was very little loss, but they then began to die quite rapidly and were all dead by the 15th day.

They were also considerably contorted as though the solution had affected the pigment cells in different portions of the body unequally. In ordinary development individual pecularities are found in eggs from different bunches i. e. those laid by different females.

These solutions seem to magnify such pecularities, and this factor must always be taken into consideration when comparing the effects produced by the solutions.

The development of different eggs in the same bunch is approximately uniform, and so the retarding influence produces nearly the same effect upon them all.

Experiment 3. Some Rana eggs in the 16 cell stage were placed in Ringer solution under the same conditions as those of Experiment 2. These remained dormant for a slightly longer period. This may have been due in part to individual differences in the two bunches of eggs, but subsequent experiments go to prove that it was partly also the result of their advance in development.

The solution seemed to have more influence over the yolk cells in these eggs and an enormous yolk plug protruded from the blastopore. The pigment was unable to cover this surface and so it remained light colored at the center, with numerous dark streaks running over it around the border. Ten individuals ($16^2/_3$ %) out of this lot were developed to the appearance of the external gills, which occurred on the 15th day. These were then preserved for further examination.

The proportion of eggs which died during development was much larger than in either of the previous experiments.

Experiment 4. A fourth lot of Rana eggs in advanced segmentation (64 cells onward) were placed in Ringer solution at the same time with those of Experiment 3, and were kept under the same conditions. The influence of the solution was more marked than in the previous cases, development was slower, and a much larger proportion died. The period of rest was nearly three hours (2 hrs. 50 mins.) and the eggs did not appear to recover as fully as in Experiment 3. There was also another significant difference. These eggs were farther advanced when placed in the solution, and yet their subsequent development was slower than that of the 16 cell eggs.

Although both were subjected to the solution at the same time, the 16 cell eggs reached gastrulation considerably in advance of the 64 cell ones.

Here again individual pecularities may partially explain this difference in rapidity of development, but is does not seem probable that this alone could occasion so great a discrepancy. None of these embryos could be reared to the formation of the external gills although every effort was made to secure that result. They died off very rapidly during early development and none lived beyond the twelfth day.

Experiment 5. A bunch of Rana eggs in which the blastopore was fully developed was obtained March 25th. A portion of these were placed at once in a 0.6 % salt solution. These lay dormant for nearly 10 hours without showing any signs of further development. They then advanced very slowly. Although the blastopore was fully developed and had begun to contract in size before the eggs were placed in the solution, this action ceased, and there was no further diminution for several days.

That this was not the result of a complete cessation of activity was proved by the fact that the embryos showed the usual amount of development elsewhere.

They elongated, the neural folds appeared and gradually fused, and the head was differentiated. Sections also showed that internal development was going forward slowly. The activity of the yolk cells alone seemed temporarily paralyzed. There was the same migration of material from the sides of the blastopore to the center of the dorsal lip, and the latter began to elongate and grow out into the tail. The failure of the borders of the blastopore to contract could not have been due, therefore, to any lack of activity in the pigment cells, but must be attributed to the sluggishness of the yolk cells. This is another evidence in favor of the view that even at an advanced stage in the formation of the blastopore, the withdrawal of the yolk cells is the first requisite for the completion of that formation, and the yolk cells are themselves the active agents of their own withdrawal.

It would be suggested also that ordinarily these yolk cells contain just about enough active protoplasm scattered amongst their yolk granules to accomplish such a withdrawal. The influence of the salt solution weakens the activity of this protoplasm, and thus renders the withdrawal impossible for the time being. Subsequently this activity may recover from the influence of the solution, and increase until it is sufficient to move the yolk cells.

Such an adaptation of vital functions to altered conditions is by no means rare.

It may be said that something of the sort always occurs when the change in conditions is not sufficient to overcome the function altogether. If the change be comparatively small, as in the present instance, the organism may be able to recover completely, and resume its normal functions.

This is shown more clearly in Experiment 12. The remainder of these eggs were allowed to develop normally for four days before being placed in the solution.

In this time they had reached an advanced stage, the blastopore being closed, the body elongated, and the anterior and posterior ends well developed. The solution produced no visible external change in their further growth, but they all weakened and died within five days.

We have thus subjected Rana eggs in different stages of development to the influence of both salt and RINGER solutions.

We find that eggs which have already begun to segment always lie dormant for a time on being placed in the solution.

The first influence of either solution is like that of a low temperature and results in a complete checking or temporary paralysis of the vital activities. The eggs recover and go on developing after a period which varies with the amount of segmentation which they have undergone previous to being placed in the solution; the more advanced the segmentation the longer the period of rest.

Subjection to either solution at a late stage of segmentation is also more likely to prove fatal. After developing normally for a time the organism is not as capable of with-standing adverse influences as when it is subjected to them before any development has taken place.

Experiment 6. Some Amblystoma eggs in the 4 cell stage were placed in the same dish with the Rana eggs of Experiment 3. (In Ringer solution.) This bunch was left with the jelly as it was found, and was not changed in any way.

The eggs developed very slowly for 17 days when the first traces of the external gills began to appear. They were thus delayed more than the eggs in 0.6 % salt solution since the latter in the same time had produced embryos with the external gills fully formed. A few of them had a very small yolk plug still protruding, but the majority appeared perfectly normal.

We have already stated that these 4 cell Amblystoma eggs were put in the same dish with the 16 cell Rana eggs, so that the conditions of development must have been exactly the same. The results, however, were very different, for while only 16⅔ % of the Rana embryos lived to the 15th day, the Amblystomas were all alive on the 17th day.

About one half of them were then preserved and the rest allowed to develop. As soon as the stomodeum opened in both Ranas and Amblystomas a marked change took place in their development.

Hitherto the yolk cells had furnished the nourishment necessary for the growth of the embryo, and everything had advanced steadily, though slowly. Now the point was reached, where the digestive canal begins to function and external food becomes necessary. Ordinarily at this stage the embryo finds enough to eat in the aquarium. These had to be kept in a clean dish for obvious reasons. An attempt was made to feed them, but nothing proved acceptable, and hence they soon began to die. Two of the Amblystoma however lived until the 24th day.

In comparing Amblystoma with Rana we must keep in mind that the latter developed faster than the former, and so could not be expected to live as long. But this does not explain the fact that all the Amblystomas lived until after the opening of the stomodeum while most of the Rana died before reaching that stage.

Experiment 7. Amblystoma eggs in both the 16 and the 64 cell stages were placed in Ringer solution in the same dish. These were also left in the original jelly untouched, but they developed more slowly than those of Experiment 6. Here again we have eggs at different stages of development, and in this instance eggs of the same species, placed simultaneously in the same solution under exactly similar conditions, and yet yielding very different results. They were necessarily taken from different bunches, but in the one case (Experiment 6) we find the Amblystomas all alive after 17 days development, and nearly all presenting a perfectly normal appearance (fig. 8). In the other case (Experiment 7) we find a much larger percent with their yolk plugs still protruding at the formation of the external gills (fig. 7), while nearly half (45 %) of them died before reaching that stage. Considerable difference could also be detected in the early development of the two bunches in the present experiment.

Experiment 8. Amblystoma eggs with the blastopore just developed were placed in Ringer solution March 27th. In these very large yolk plugs were left protruding, even at the end of 16 days development, at which time also nearly all (90 %) of them were dead.

By comparing these eggs with those in Experiment 6 several interesting facts appear.

These eggs had developed normally until the blastopore was fully formed and its borders had begun to contract. But on being placed in Ringer solution the action, begun and carried so far forward, was not completed, and the yolk plug remained very nearly of its original size. On the contrary the 4 cell eggs, in which even segmentation was scarcely commenced, formed a blastopore at the proper time, and most of them completely closed it.

Again the two lots of eggs were placed in the solution within an hour of each other, but at the end of ten days the 4 cell eggs were as far developed as the others. At the end of 16 days they were much farther developed, notwithstanding the fact that the eggs of the present experiment had so much the start of them. The difference between the 4 cell stage and gastrulation represents about three days development, and yet this comparatively large handicap was overcome within 10 days time. Evidently in this instance the influence of the surrounding medium was greatly modified by differences in the stage of development at which the egg was subjected to it's action.

Experiment 9. Chorophilus eggs, unsegmented, were placed in Ringer solution. These eggs did not seem to lie dormant at all, but continued developing at once. The influence of the solution upon their development, however, was very marked in other ways.

Gastrulation was much delayed and the blastopore, when formed, occupied fully one half the surface of the egg. As the embryo began to elongate this yolk mass, which was out of all proportion to the size of the embryo, was left free, and occupied the whole of the ventral surface. In many cases the embryo was simply coiled around the yolk and presented an appearance very similar to that in the eggs of bony fishes. This free yolk became loose and mealy and portions of it sloughed off from time to time.

For this reason the embryos died rapidly, and none of them reached the closure of the neural folds. For the same reason it was found absolutely impossible to preserve any of the eggs so that they could be sectioned, though many different methods were attempted. Accordingly it was determined to try these eggs in a weaker solution, with the results given in Experiment 12.

Experiment 10. Chorophilus eggs in the 4 cell stage were placed in the same dish with the 16 cell Rana eggs of Experiment 3, and the 4 cell Amblystoma eggs of Eperiment 6. These eggs had all been taken from the same pool in close proximity to one another, and by keeping them thus in the same dish it was possible to make intelligent comparisons of the relative influence of the solution upon them. The Amblystoma eggs were retarded most in their development, and the Chorophilus eggs the least. The former remained inactive for some time after being placed in the solution while the latter seemed scarcely to stop at all. The Rana eggs were inactive for a time, but not as long as the Amblystomas.

On the other hand the ultimate effect of the solution was exactly the reserve of this. The Amblystoma eggs recovered their activity slowly, but seemed to recover it fully, and continued developing until they became quite normal embryos with fully developed external gills.

In strong contrast with these the Chorophilus eggs, which at first were scarcely influenced by the solution, in later development were so affected by it that none of them could be reared even to the closing of the neural folds.

In this particular, also, the Rana eggs occupied the middle ground. They were more affected than the Amblystoma eggs, and not as much as the Chorophilus.

Ringer solution, therefore, retards most those eggs whose development is least rapid, while its ultimate influence is greatest upon those eggs which develop most rapidly.

Experiment 11. Some Chorophilus and Amblystoma eggs, each in the 64-cell stage, were placed together in a 0.6 % salt solution on March 28th. Again the Amblystoma eggs remained dormant for some time (in this case 5 hours) while those of Chorophilus continued developing with scarcely a break, but very soon a marked difference between the two species became apparent.

Both kinds of eggs were at the same stage when placed in the solution, and according to the normal rate already given the Chorophilus eggs should have developed much more rapidly than those of Amblystoma. Such was not the case, however; they kept along very evenly for a day, and then the Amblystoma eggs gained slowly on the others and eventually reached gastrulation nearly 24 hours ahead of them.

The Chorophilus eggs which should have gastrulated within 12 hours did not reach that period until the 5th day, while the Amblystoma eggs that should have formed the blastopore on the second or third day were only delayed till the fourth day.

Moreover the Chorophilus eggs were very much shrunken and contorted, especially around the head and anterior end, this portion of the body being covered with ridges and small papillae.

The yolk plugs were very large and showed the same pigmentation as has already been noticed in the case of Amblystoma and Rana, though not quite so clearly.

These Chorophilus eggs lived for 15 days and in that time had not completed their gastrulation. The Amblystoma eggs on the contrary, developed with some loss up to the formation of the external gills when the few that were left were preserved.

In this experiment, therefore, the effect of the same solution under the same conditions was very different in the two kinds of eggs, and was the same as that obtained in the previous experiment.

Experiment 12. In connection with the fact that it was found so difficult to develop the Chorophilus eggs in any of the solutions beyond the gastrula period, the following experiment is of special significance.

It was suggested by the results obtained by DAVENPORT and NEAL in the »Acclimatization of organisms to poisonous chemical substances« (9).

Some unsegmented eggs of Amblystoma, Rana and Chorophilus were placed in a 0.05 % salt solution on March 29th.

They were allowed to remain in this very weak solution for 24 hours. At which time the solution was poured off and replaced by one of double the strength (0.1 %). Subsequently every 24 hours the strength of the solution was increased 0.1 % until it reached 1 %. All the eggs developed normally without any indication of the free yolk plug. Some of the Chorophilus eggs hatched out in four days, when the solution had a strength of 0.3 %. They lived until the reserve food material of the yolk was used up, when they all died, having refused any other food. The last one died on the 10th day when the solution had a strength of 0.9 %.

The Rana eggs hatched in 7 days (0.6 % sol.) and some of them lived for 6 days afterward, when the few that remained were preserved for examination.

The Amblystoma eggs did not hatch until the 12th day, and then lived 4 or 5 days longer in a 1 % solution.

We thus have embryos living and swimming about freely in a solution of sufficient strength to kill them if they had been placed in it immediately.

We know from our own experiments that the influence of the salt solution is negatively, rather than positively injourious i. e. it does not directly injure the protoplasm but simply hinders or prevents it from performing its normal function. This preventive influence has more effect upon the protoplasm than upon the nucleus and when it becomes sufficiently strong to overcome the functional activity of the latter the organism dies.

The present experiment teaches that the ability of both nucleus and protoplasm to withstand this influence in any particular case is capable of education and can be increased by being gradually exercised. This development can be carried so far as to enable the organism to easily overcome influences that would otherwise quickly prove fatal. There seems to be differences between the three species in their capacity for such development. In ordinary life many adverse influences, such as stagnation of the water or the presence of any salt or mineral in solution, come on gradually, and this capacity on the part of the embryo for increasing its ability to withstand such influences must prove of great service, and often times prevent a fatal issue.

In reviewing the teaching of these experiments the first general fact which they indicate is that »the extraordinary delicacy with which the frog's eggs respond to slight variations in the surrounding medium« (Hertwig) is closely connected with rapidity of development.

We can easily see that this must be so, for the rapidity varies with the relative amount of active protoplasm which the egg contains. Any given influence, therefore, will produce very different results according as it is exerted upon eggs having a large quantity of such protoplasm, or upon those possessing only a meagre amount.

A second fact is one already stated by Hertwig. The »lowering of the energy in the different parts of the egg is not equal but regulates itself according to the proportion in which the active protoplasm substance and the more passive nourishment material are contained in the egg cavity«. This fact appears in every experiment with the three species now being considered as plainly as with the two species of Rana upon which Hertwig experimented.

A third fact follows in logical sequence i. e. the effect produced by the surrounding medium does not depend upon the absolute amount of active protoplasm which the egg contains, nor upon the relative amount compared with its size, but upon the relative amount contained in the animal half compared with the vegetative half. The large Amblystoma eggs contain relatively a smaller amount of this active substance than the small Chorophilus eggs. Consequently their ordinary development is much slower, and they are retarded more by the restraining influence of the same medium. But there is less difference between their animal and vegetative cells in the amount of active protoplasm which each contains than is found in the more rapidly developing Chorophilus eggs. In consequence of this, the influence of the solution is more nearly equal upon the two kinds of cells. The both lie dormant for a time, and although the animal cells recover first they do not develop rapidly enough to prevent the vegetative cells from recovering in their turn and following them at a comparative rate approximately normal.

On the contrary, in the eggs of Chorophilus there is a great difference in the amount of active protoplasm contained in the animal and the vegetative cells. Consequently when brought under the influence of a restraining medium there is an increased difference in the effects produced upon the two kinds of cells. The animal cells have so much activity that they seem scarcely restrained at all, but continue to develop at nearly the normal rate. But the vegetative cells, possessing only a little active protoplasm are restrained so much that they can scarcely continue at all, or may even be compelled to lie dormant for a while. In this way differences are produced which do not exist in normal development. These differences increase rapidly, and in a short time become so great as to result in the death of the embryo.

The Amblystoma egg, therefore, possesses a bulwark of safety in the more even distribution of its protoplasm, and is able thereby to overcome influences which result disastrously to its more rapidly growing relative. But at the same time the sluggish development, which is the result of this more even distribution, increases the period of exposure to danger, and a great many Amblystoma eggs usually die in the jelly.

Just subsequent to the experiments here given a dozen bunches of Amblystoma eggs were found in a boggy field at the edge of the woods. The water was nowhere more than 6 or 8 inches deep, and was so thoroughly impregnated with iron as to tinge the soil and herbage a deep rust color.

It did not seem as if any eggs could develop in such a medium, but subsequent examination proved that they actually did. Some of the bunches were taken to the laboratory and produced perfectly healthy embryos. A visit was made to the bog at about the right time and the eggs were found hatching, apparently with no more than the ordinary loss. No doubt they produced an average number of adults provided the water did not dry up prematurely. This furnishes a good example of the protection afforded against influences which must arise quite frequently in natural development.

Again we are able to infer that the amphibian embryo can accommodate itself to adverse circumstances at the very beginning of its development more readily than it can readjust its vital powers after a sudden change during later growth. The older it gets, and the farther it is developed normally the less becomes its ability to change into something abnormal.

This truth has been recognized for a long time as applying to the adult life of an animal, and it seems to be even more applicable to embryonic stages of development. According to the experiments here recorded, eggs at stages as near together as the unsegmented and the 64-cell stage show a difference in their ability to overcome the influence of the solutions. This becomes very marked in comparing eggs subjected to the solutions at the beginning and at the close of segmentation. We have here, perhaps, a stronger evidence than that given by HERTWIG, of the great delicacy with which the amphibian embryo responds to variations in the surrounding medium.

These experiments also bear significant testimony in another direction. The early differentiation of cells as to function, and their consequent interdependence has been admirably worked out by eminent investigators in the recent morphological examination of different embryos (37 and 48), but it scarcely admits of physiological proof under normal conditions.

Under these abnormal conditions, however, a comparison of experiments indicates quite clearly that in the unsegmented egg there is very little differentiation. If there be any at all it is a very plastic one, and accommodates itself easily to changed conditions. But as soon as segmentation begins differentiation commences also, and the egg acquires thereby definite regional distinctions.

These increase with advancing development and at the 64-cell stage they become sufficient to hinder the egg perceptibly in accommodating itself to a new environment.

In the blastula stage they furnish a serious obstacle to any such accommodation, and by the time the blastopore is fully formed they have become so well established that they cannot be altered, and consequently the organism must die under the influence of any serious change in its environment.

HERTWIG has clearly shown that the cell material which is used in these embryos for the origin and development of the dorsal organs and the tail is differentiated in a very different manner from that which occurs in normal development.

The same truth has been shown in the present experiments when discussing the fate of the blastopore. It is this readjustment of differentiation which proves so difficult for the embryo, since material which has been gradually separated for a particular purpose, or at least for the accomplishment of that purpose in a particular way, must be used for a different purpose or for its accomplishment in an entirely different way. This subject of differentiation during development, and of the potence of the early blastomeres, has been eminently worked out by WILSON (49) and MORGAN (38) in very recent publications. They both frankly state that at present we do not know how such differentiation is brought about. MORGAN »thinks there are sufficient reasons to conclude that the power of differentiation lies within the egg itself, and does not depend directly on external stimuli« (38, pag. 134).

The present experiments would seem to strengthen this conclusion, and to show, in addition, that the power may be strong enough to overcome considerable adverse external influence. In spite of the restraining influence of the salt solution the differentiating power asserted itself and ultimately produced normal embryos, when the eggs were subjected to the solution before they had begun to segment.

Of course the more rapid the development the sooner will the differentiation be accomplished, and we may reasonbly infer the more rigid will it be. Such eggs, therefore, as those of Chorophilus could not accommodate themselves as readily to a changed environment as those of Amblystoma in which the differentiation takes place more slowly, and thereby allows time for readjustment.

Westfield, Mass. U. S. A.

Zusammenfassung.

Diese Experimente ergaben, in Hinsicht auf die drei erwähnten Amphibienspecies, die folgenden Thatsachen:

1) Sowohl einfache Salzlösung wie gemischte Salzlösung (Ringer-Lösung, s. pag. 627, Anm.) üben, je nach ihrer Koncentration, einen hemmenden Einfluss auf die Entwickelung aus. In einer 1 %igen Salzlösung wird diese Einwirkung stark genug, um die Entwickelung völlig zu verhindern.

2) Entsprechend den Unterschieden der Aktivität der Dotterzellen und der pigmentirten Zellen (Bildungszellen) werden erstere stärker gehemmt als letztere. In Folge dessen ist alle Entwickelung, die irgendwie von den Dotterzellen abhängt, abnorm verzögert. In dieser Weise können Differenzen hervorgebracht werden, welche genügen, um Absterben hervorzurufen.

3) Aus demselben Grunde werden auch verschiedene Theile einer jeden Zelle verschieden betroffen. In Folge dessen sind die karyokinetischen Figuren nicht alterirt und daher gut zu sehen.

4) Je schneller eine Species sich entwickelt, desto geringer ist der unmittelbare Effekt des hemmenden Einflusses auf entsprechenden Stadien;

5) desto schlimmer ist aber der Endeffekt.

6) In Eiern derselben Species auf verschiedenen Stadien der Entwickelung ist die Wirkung der Lösung um so größer, je vorgeschrittener die Entwickelung ist.

7) Die Fähigkeit von Amphibieneiern, derartigen Einwirkungen zu widerstehen, kann durch Gewöhnung bis zu einem Grade gesteigert werden, dass das Ei in einer Umgebung zu leben vermag, in welcher es sonst augenblicklich zu Grunde ginge.

8) In beiderlei genannten Lösungen wird Pigment in situ in den Dotterzellen entwickelt, bis die Oberfläche des Dotterpfropfes fast ebenso dunkel erscheint als das Ektoderm.

9) Bei Amblystoma bildet sich ein temporärer Anus am vorderen Ende des Blastoporus. Der Neuroporus nimmt eine Zeit lang an dieser Öffnung Theil. Mit fortschreitender Entwickelung durchwandert der Anus successive den ganzen anteroposterioren Durchmesser des Blastoporus, und der dauernde Anus des erwachsenen Thieres bildet sich am hinteren Ende des Blastoporus.

10) Auf der Rückenfläche der Amblystomalarve erscheinen an der Verschlussstelle der Neuralfalten Cilien, und breiten sich allmählich auf dem ganzen Ektoderm aus. Der stärkste Strom, den diese Cilien erzeugen, geht über die Stelle der späteren Kiemen.

11) Diese Cilien veranlassen (wie bekannt) eine rotirende Bewegung der Larve innerhalb der Eihüllen. Unter Bedingungen, welche diese Bewegung verhindern, starb die Larve.

Diese thatsächlichen Ergebnisse erlauben die folgenden allgemeinen Schlüsse:

1) Es besteht ein inniger Zusammenhang irgend welcher Art zwischen Pigmentbildung und physiologischer Aktivität. In beiden Lösungen wurde Pigment in der unmittelbaren Nähe von denjenigen Zellen oder Zelltheilen gebildet, welche physiologisch sehr thätig waren.

2) Die Entfernung der Dotterzellen ist ein primäres Erfordernis für den Verschluss des Blastoporus. Dieselbe erfolgt durch die funktionelle Thätigkeit der Dotterzellen selbst; wird sie irgendwie verhindert, so bleibt der Blastoporus offen.

3) Die Präcision, mit welcher Amphibieneier auf geringe Veränderungen des umgebenden Mediums reagiren, hängt eng zusammen mit der Entwickelungsgeschwindigkeit. Je größer diese ist, desto feiner ist die Reaktionsfähigkeit.

4) Die Wirkung von Salzlösungen hängt weder von dem absoluten Betrag an aktivem Protoplasma ab, welchen das Ei enthält, noch von dem relativen Betrag in Hinsicht auf die Größe des Eies. Eher hängt sie ab von der relativen Menge, die in den animalen Zellen im Vergleich mit den vegetativen enthalten ist, d. h. von der Relation zwischen aktivem Protoplasma und passivem Nährmaterial in den einzelnen Zellen.

5) Eine gleichmäßige Vertheilung aktiven Protoplasmas hat eine träge Entwickelung zur Folge und verlängert so die Zeit der Gefährdung des Eies. Dafür entschädigt dieselbe vielleicht durch den Schutz, den sie gegen schädigende Einflüsse im umgebenden Medium gewährt.

6) Die physiologischen Reaktionen dieser Amphibienembryonen gegen die Lösungen zeigen, dass eine funktionelle Zelldifferenzirung in wenig segmentirten Eiern nicht existirt, sondern eher ein Ergebnis allmählicher Entwickelung darstellt.

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Explanation of Plate X and XI.

All the figures were drawn with a Camera lucida.

Figs. 1 to 9 are serial surface views of Amblystoma eggs and embryos developed in 0.6% salt solution: the eggs being unsegmented when placed in the solution.

Fig. 1. Surface view of inferior hemisphere of a living egg showing first beginning of the blastopore invagination after being in the solution 72 hours. Magnified 36 diams.

Fig. 2. Surface view of inferior hemisphere of a living egg which had remained 47 hrs. in a 0.4% solution. Magnified 36 diams.

Fig. 3. Surface view, posterior, of living egg 103 hrs. in 0.6% salt solution. Magnified 36 diams.

Fig. 4. Surface view of living embryo (dorsal) 123 hrs. in the solution. Showing formation of the neural folds and groove. Magnified 36 diams.

Fig. 5. Surface view of freshly killed embryo just at the closing of the neural folds, 140 hrs. in the solution. Magnified 36 diams.

Fig. 6. Surface view (lateral) of freshly killed embryo 11 days in the solution. Showing posterior elongation of dorsal lip of blastopore and large free yolk plug. Magnified 36 diams.

Fig. 7. Surface view (lateral) of preserved embryo 17 days in the solution, showing external gills and balancer. The free yolk in this embryo was extraordinarily large. Magnificed 24 diams.

Fig. 8. Surface view (lateral) of preserved embryo 20 days in the solution, showing closure of the blastopore, and almost entire disappearance of all traces of the free yolk plug. This embryo is essentially normal in all its external features.

Fig. 9. Surface view, enlarged, of posterior end of an embryo 103 hrs. in a 0.4% solution, showing temporary anus and comparative size of yolk and pigment cells. Magnified 52 diams.

Fig. 10. Surface view (lateral) of an embryo showing abnormal separation of the posterior portion of the dorsal organ from the yolk plug.

Figs. 11—14 are sections of the same series showing formation and gradual closure of the blastopore. All from 0.6% solution.

Fig. 11. Median section of blastula, showing segmentation cavity and the beginning of gastrulation.

Fig. 12. Median vertical section of posterior end of embryo showing pigment layer over surface of the yolk, and thickened dorsal lip of the blastopore 120 hrs. Magnified 50 diams.

Fig. 13. Similar section 40 hrs. later in development showing partial withdrawal of the yolk plug and downward growth of the dorsal lip of the blastopore.

Fig. 14. Similar section 32 hrs. later in development showing complete withdrawal of yolk plug and formation of permanent anus.

Figs. 15 and 16 are outline sketches of normal Amblystoma embryos, showing currents generated by the cilia. Fig. 15 just after the closing of the neural folds and Fig. 16 12 hrs. later.

Figs. 17—20 are median longitudinal sections of Amblystoma embryos, showing method of closure of the blastopore, and the relation between the withdrawal of the yolk plug and the shape of the archenteron.

Fig. 17. Median section of an egg developed 91 hrs. in a 0.6 % salt solution, showing very large, free yolk plug and temporary anus just beneath the dorsal lip of the blastopore.

Fig. 18. Similar section of an egg developed 108 hrs. in a 0.6 % salt solution, showing elongation of embryo, uniform depth of the archenteron and enlargement of dorsal lip of blastopore.

Fig. 19. Similar section after 140 hrs. development, showing change in shape of Archenteron due to partial withdrawal of yolk plug.

Fig. 20. Similar section after 6 days development in 0.5 % solution, showing further change in shape of Archenteron, almost complete withdrawal of yolk plug, and formation of vertebral somites and brain.

Fig. 21. Similar section from normal embryo at about the same period in its development showing comparative shape of Archenteron.

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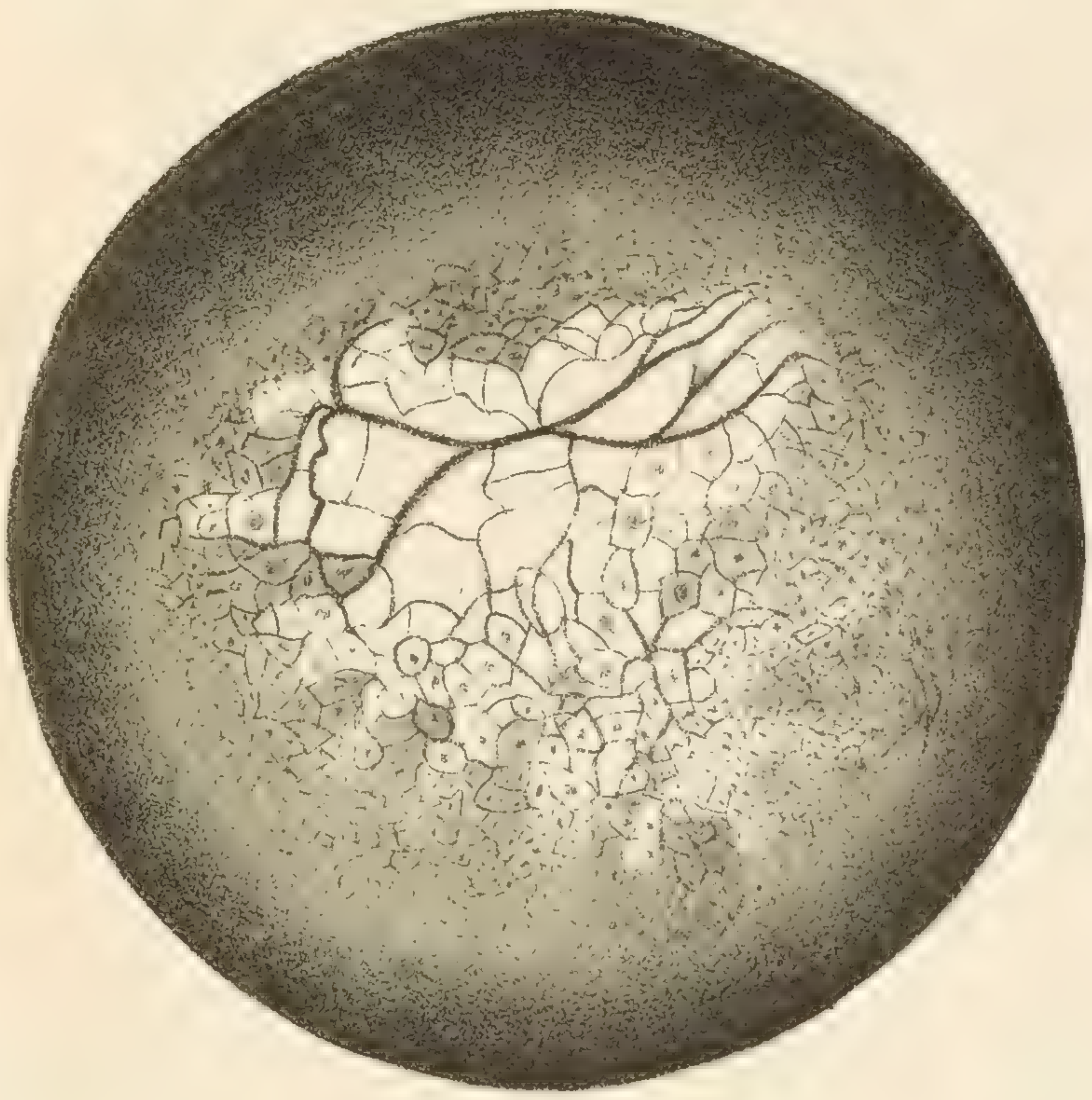

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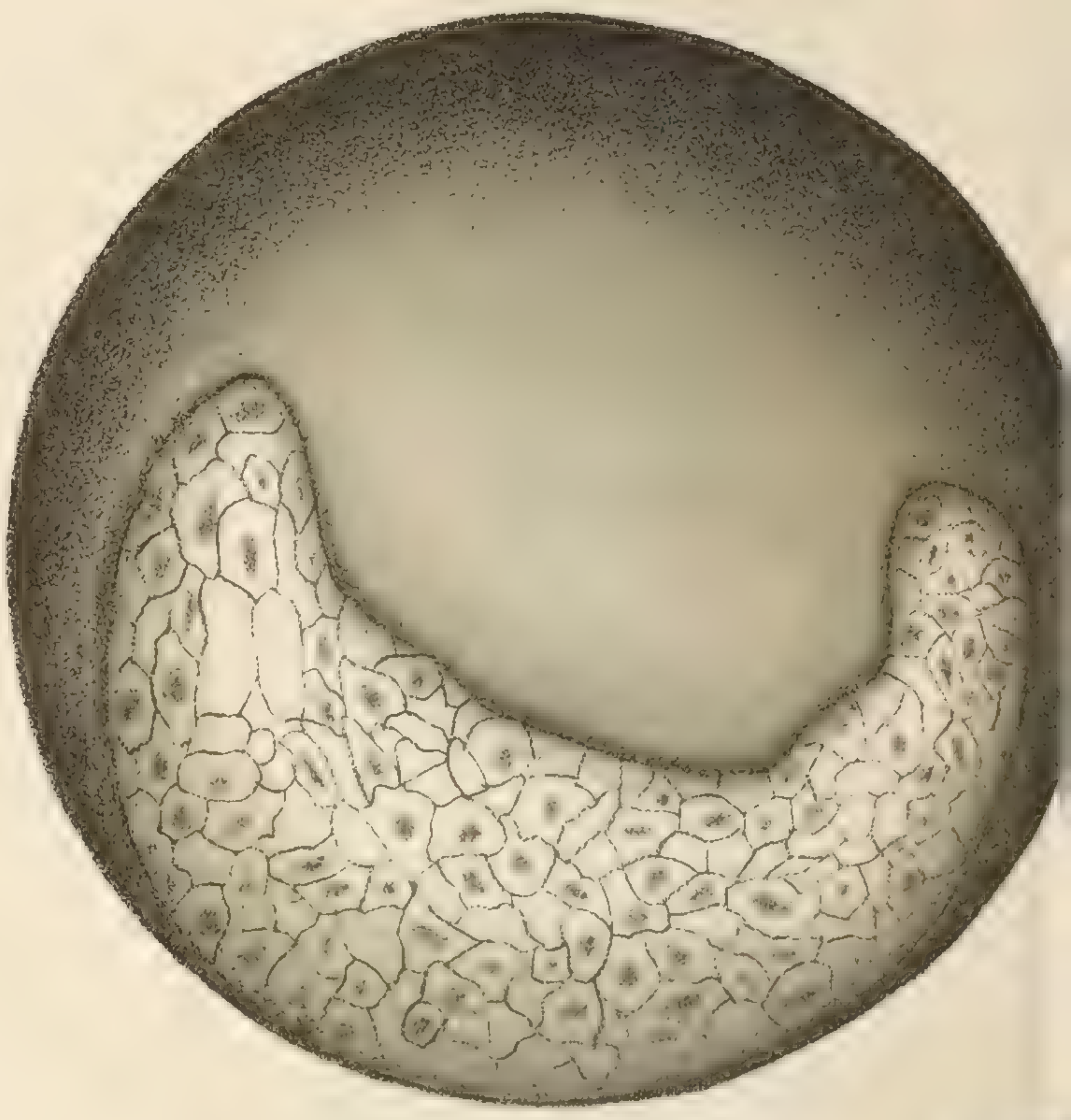

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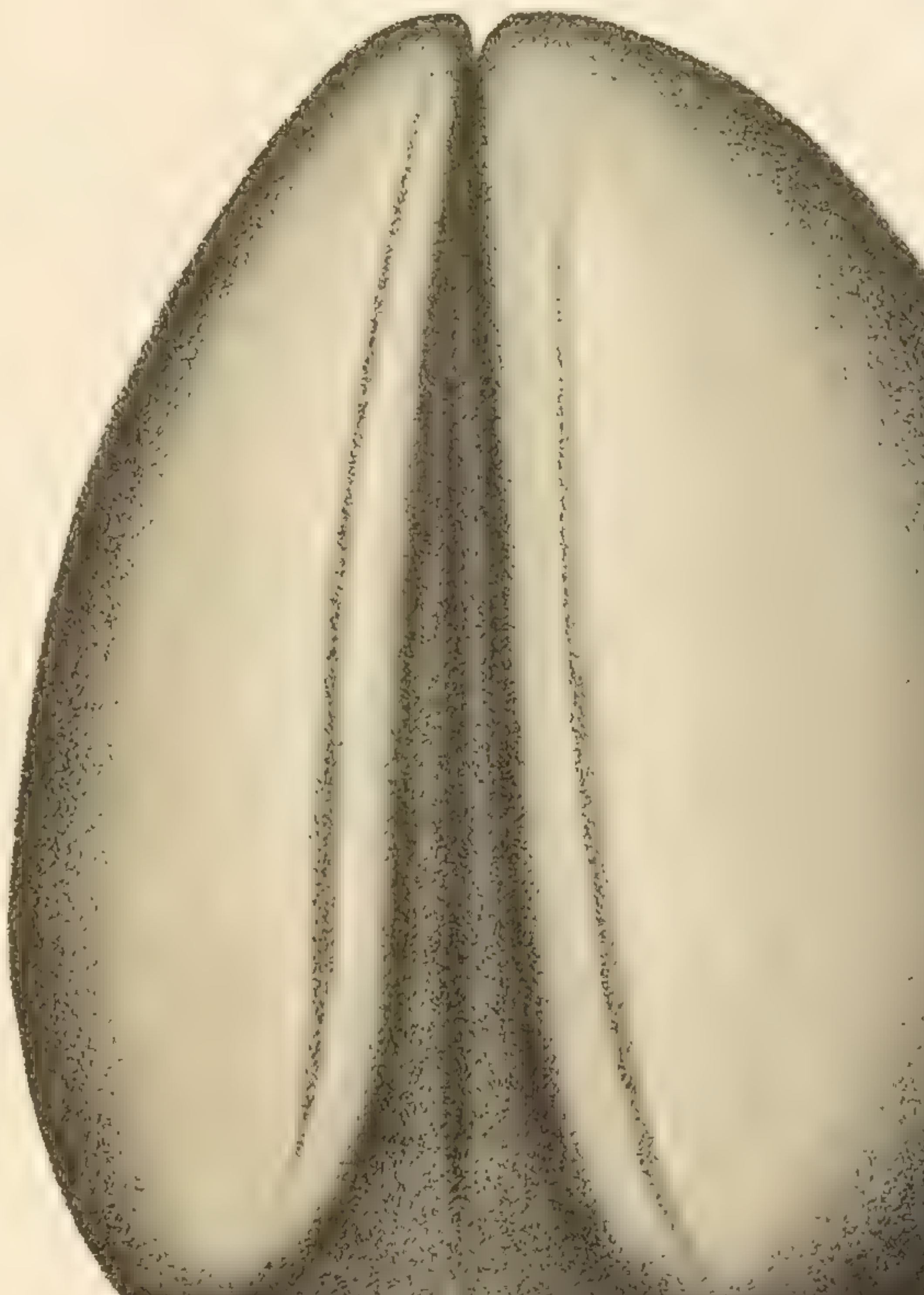

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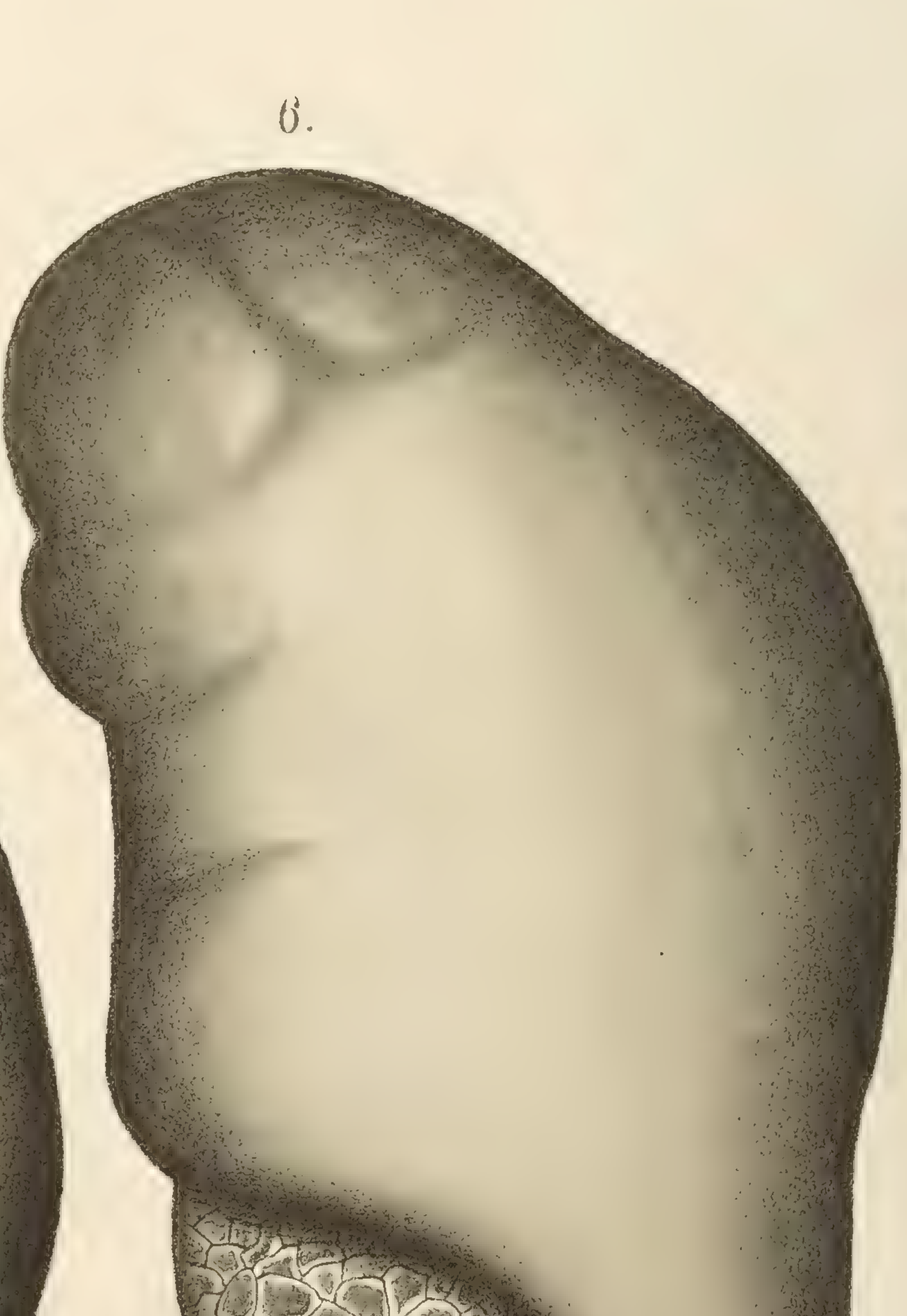

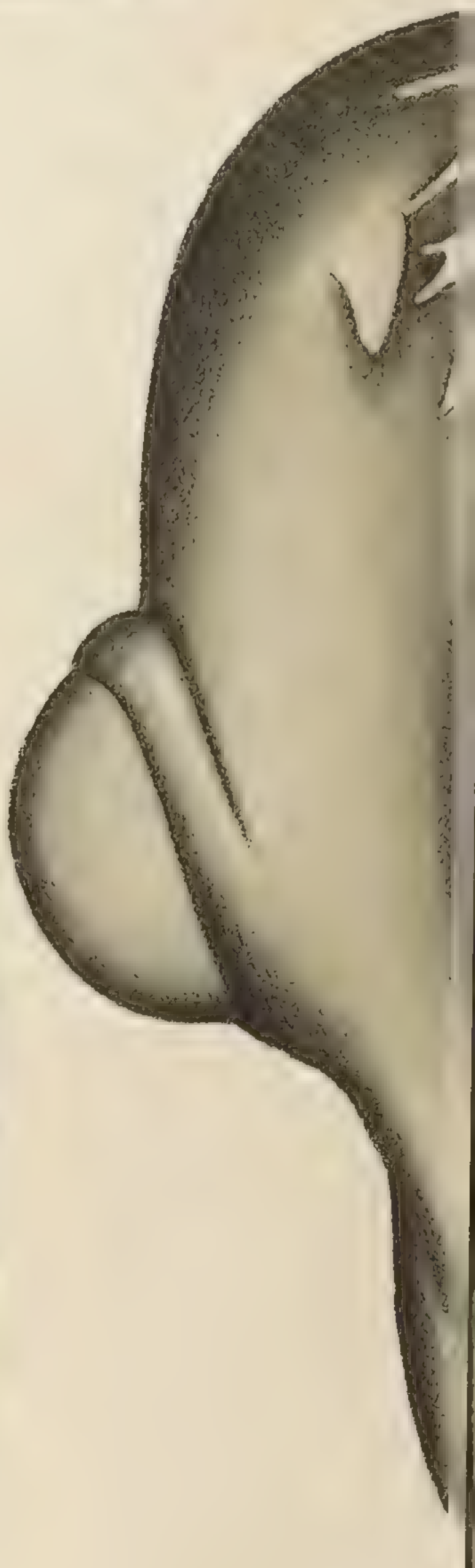

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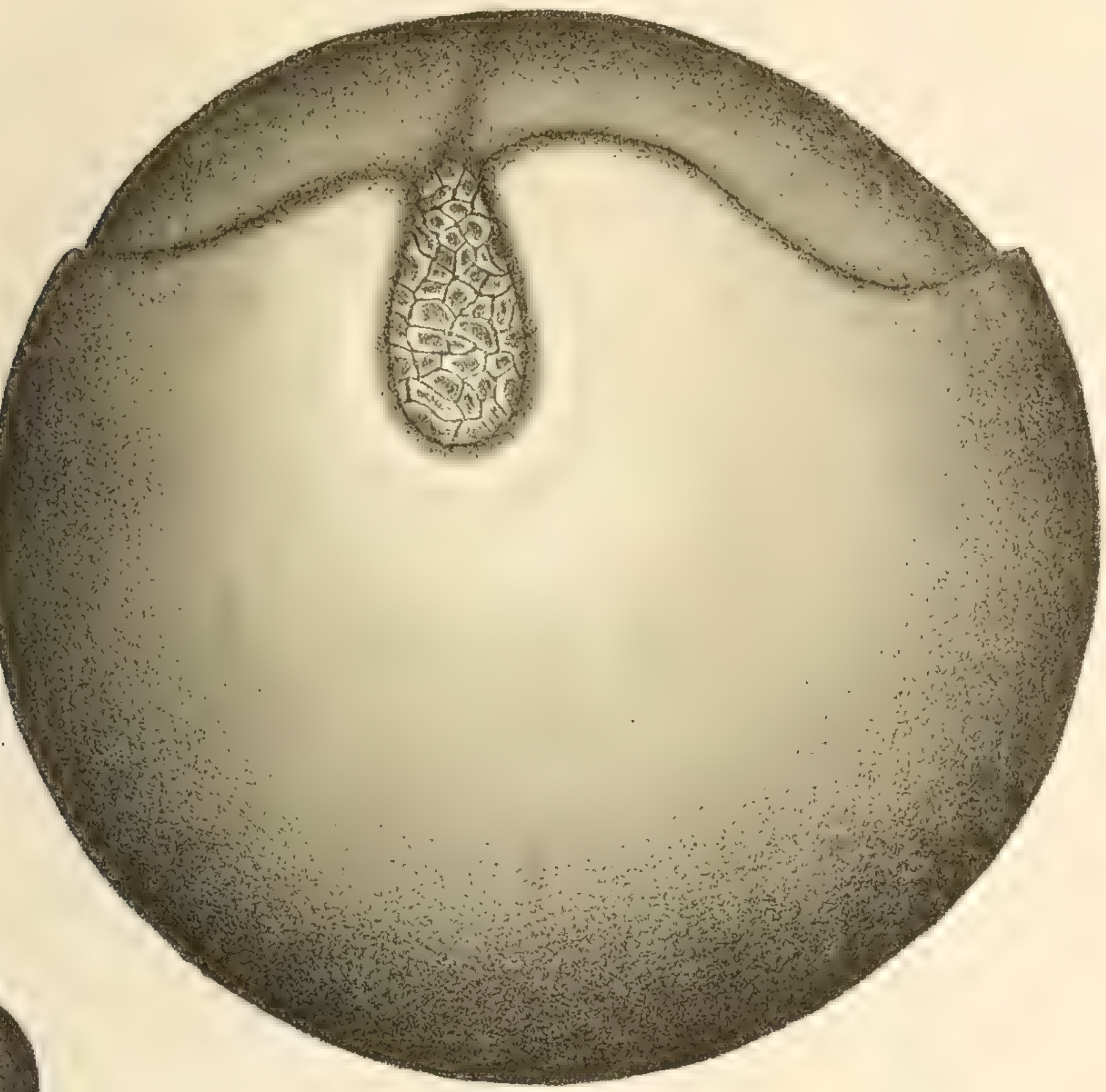

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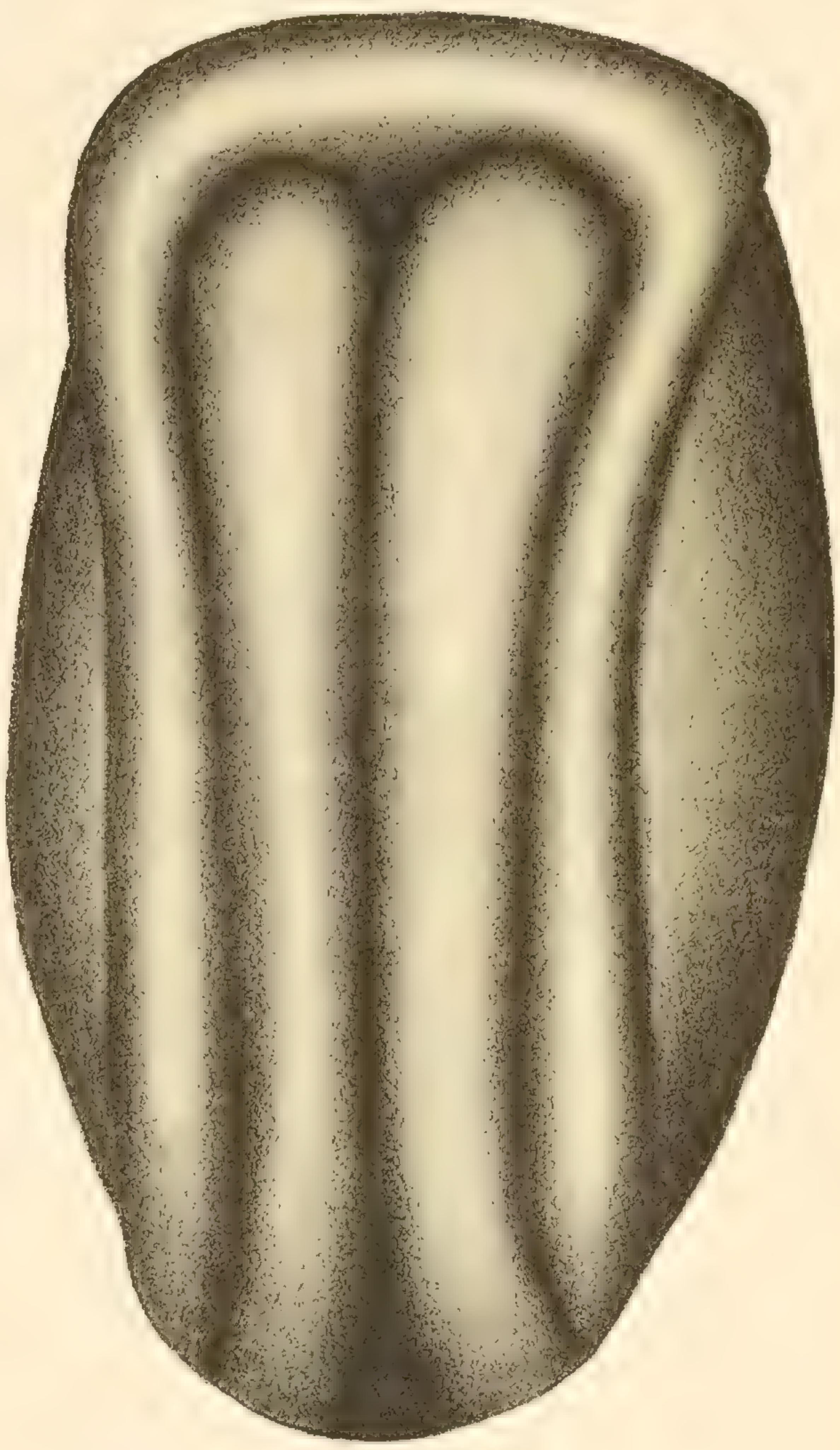

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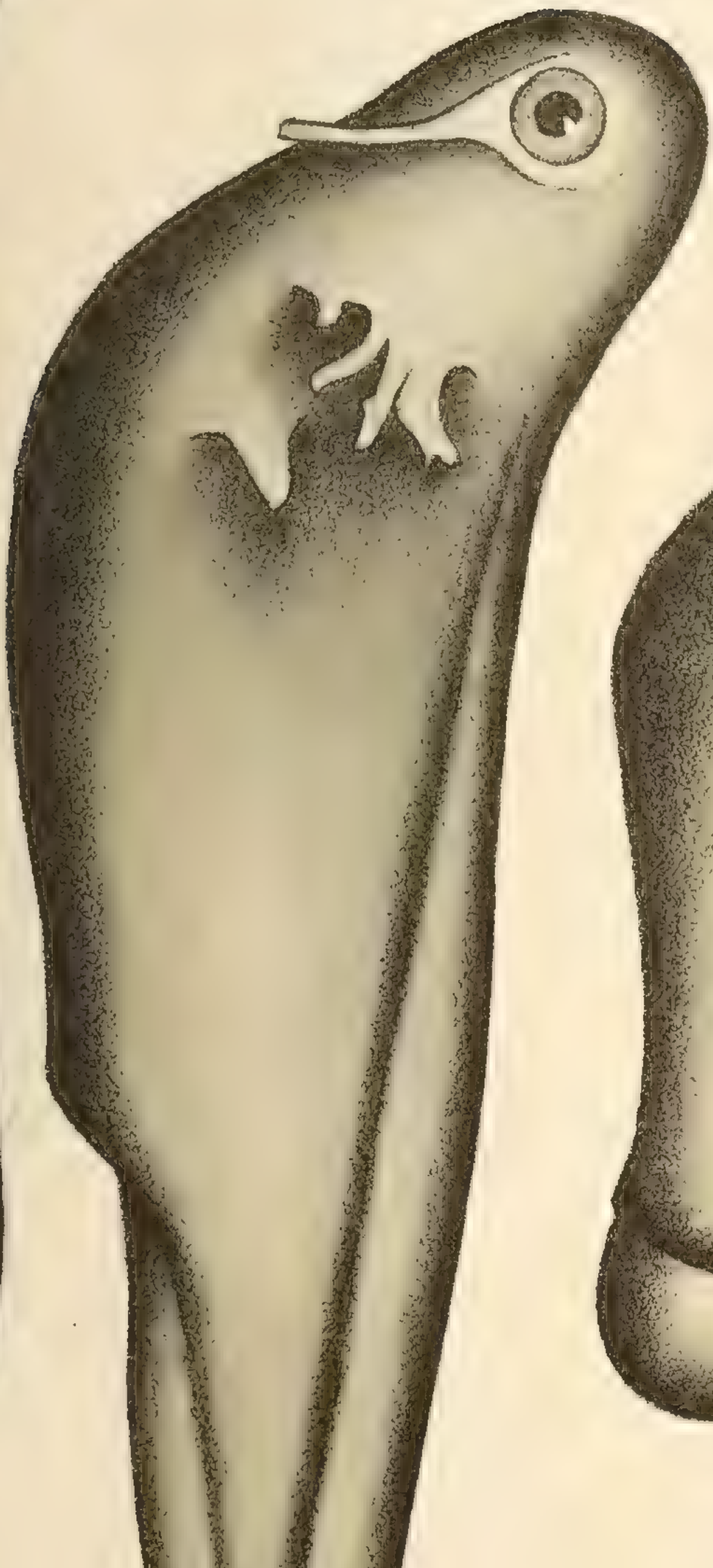

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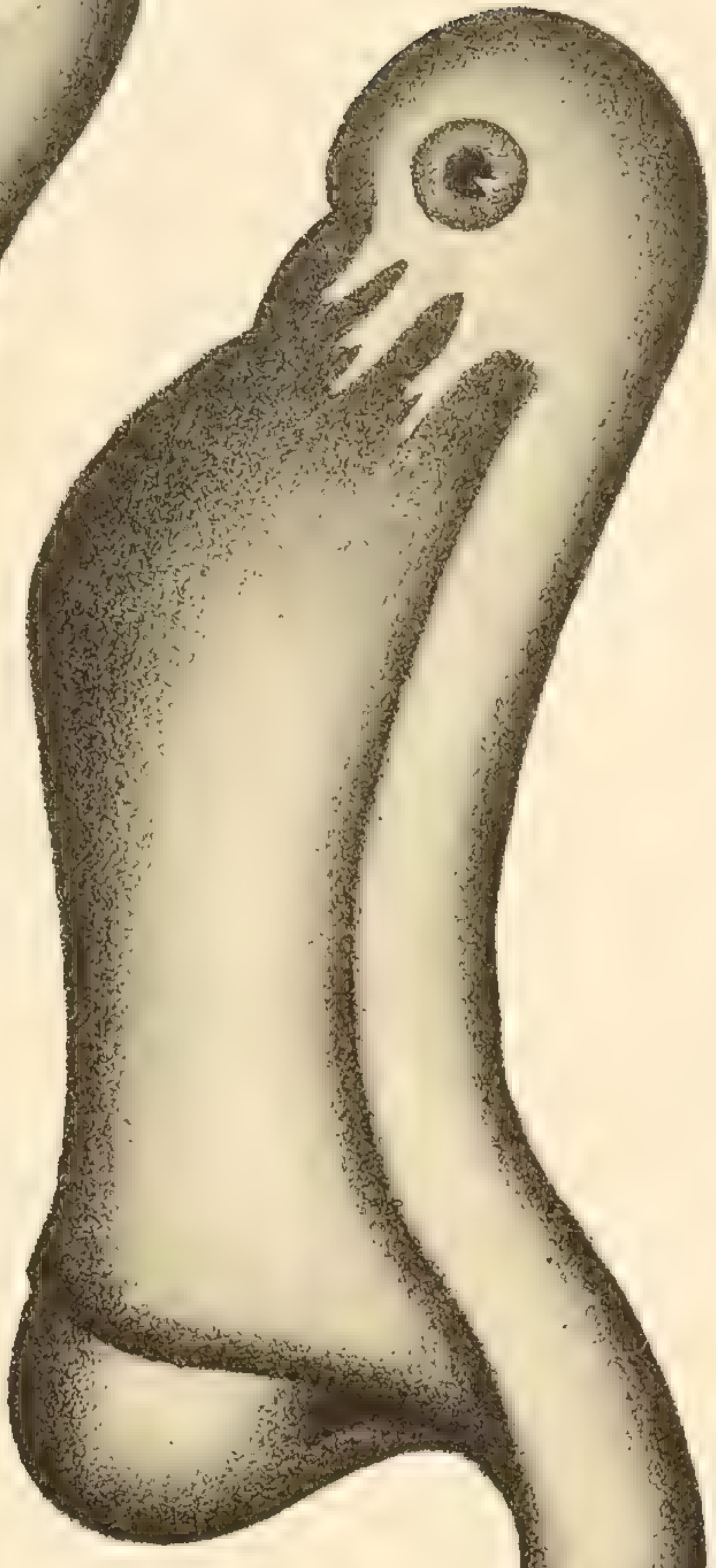

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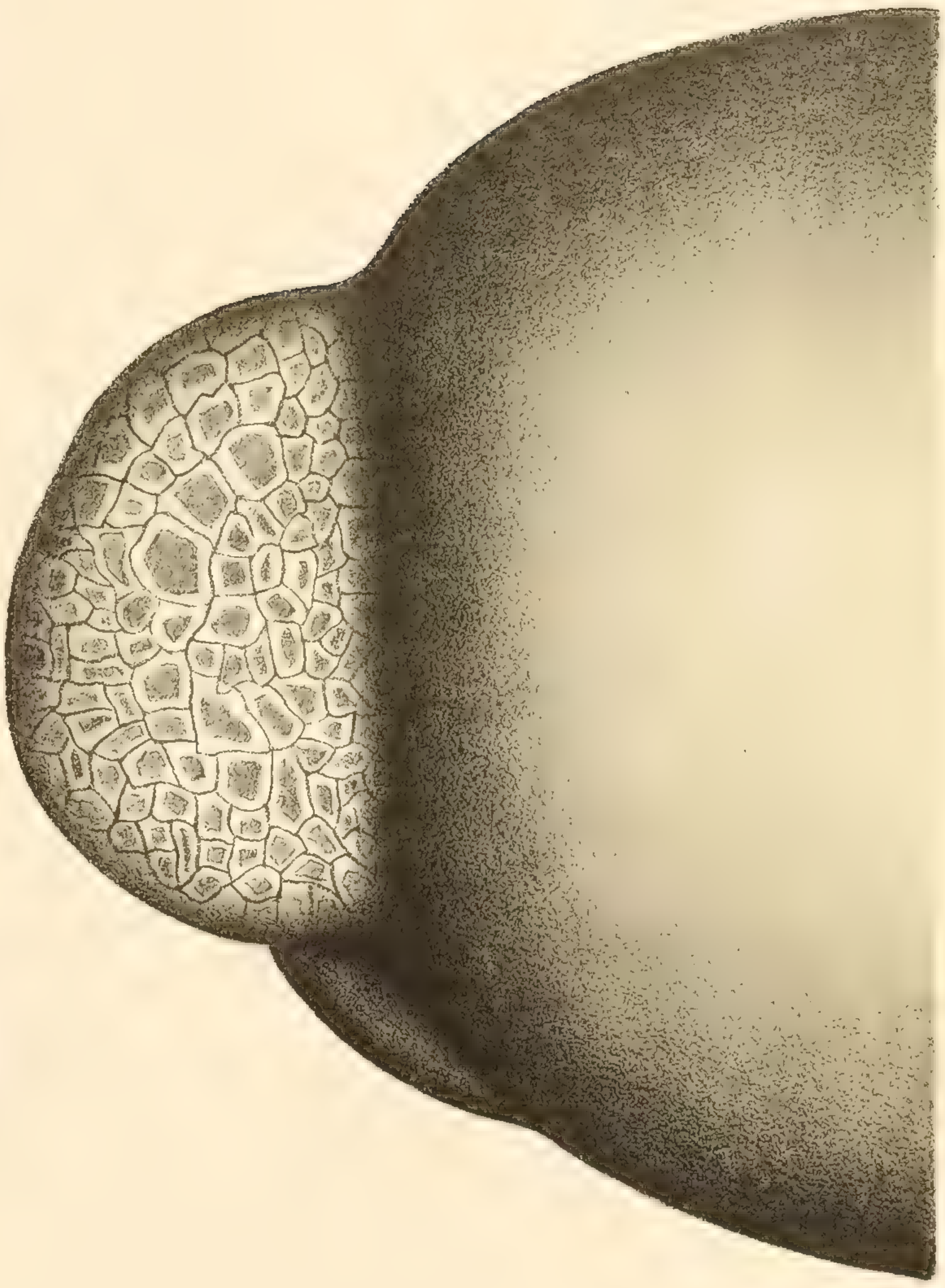

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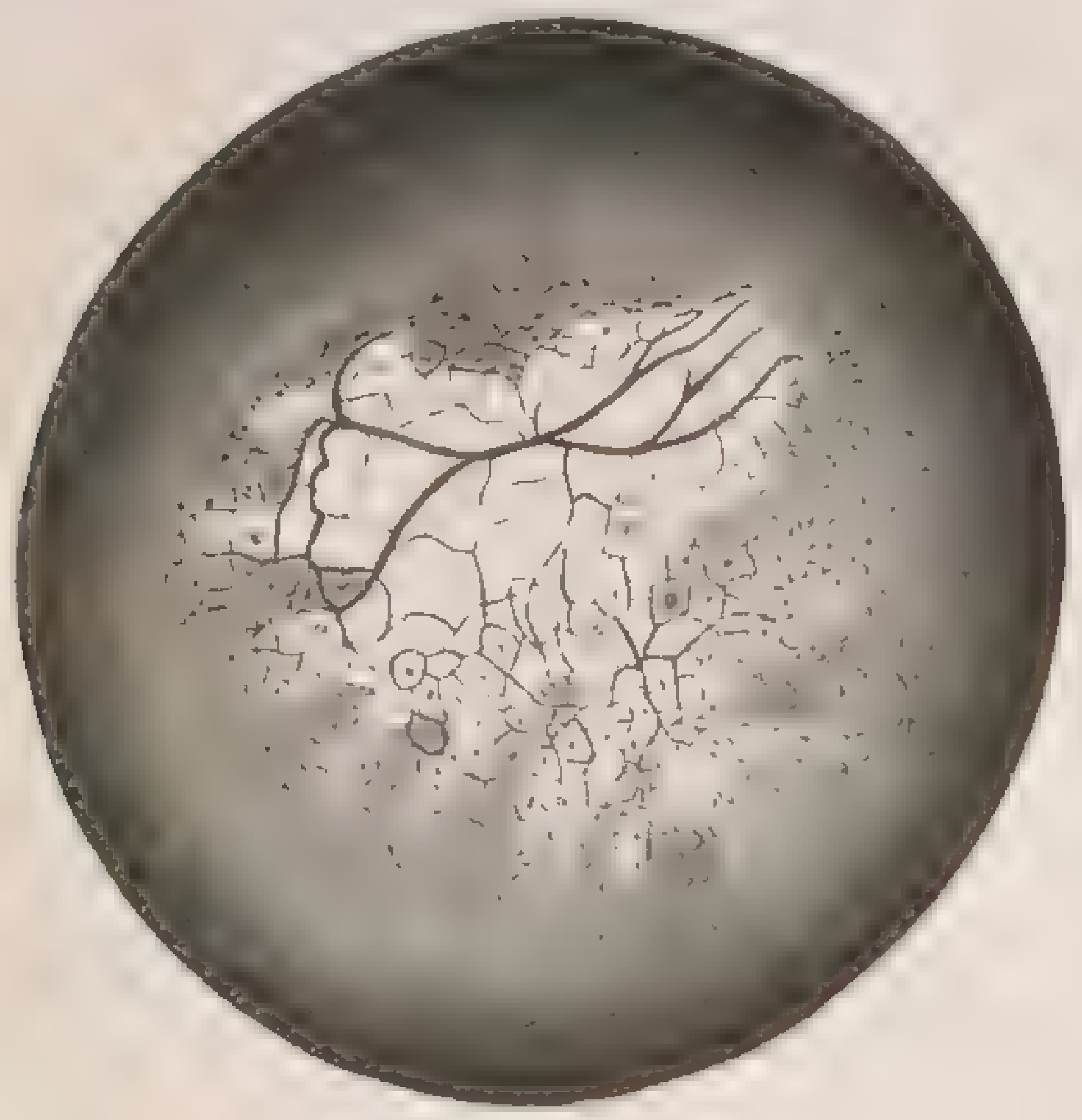

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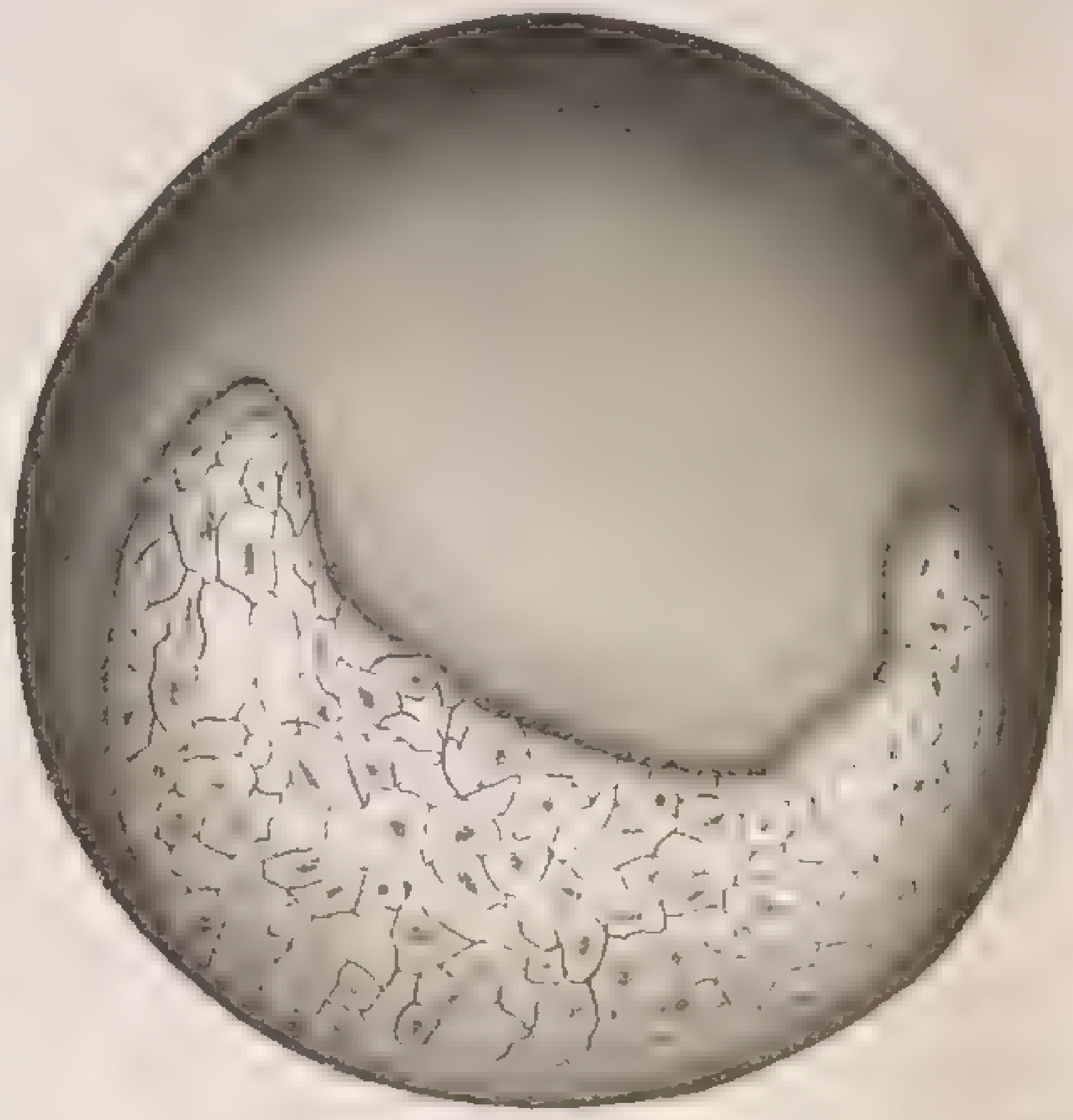

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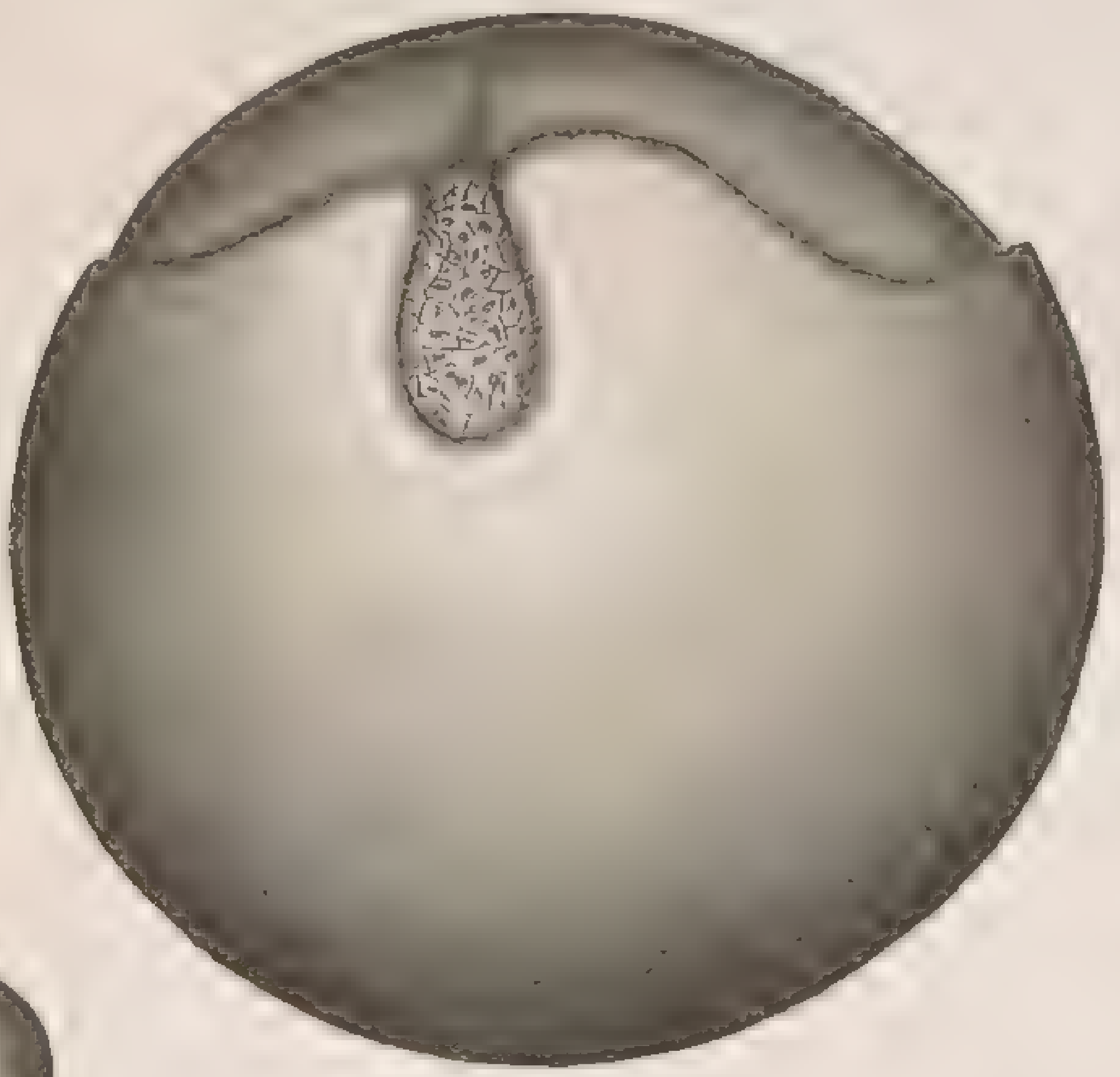

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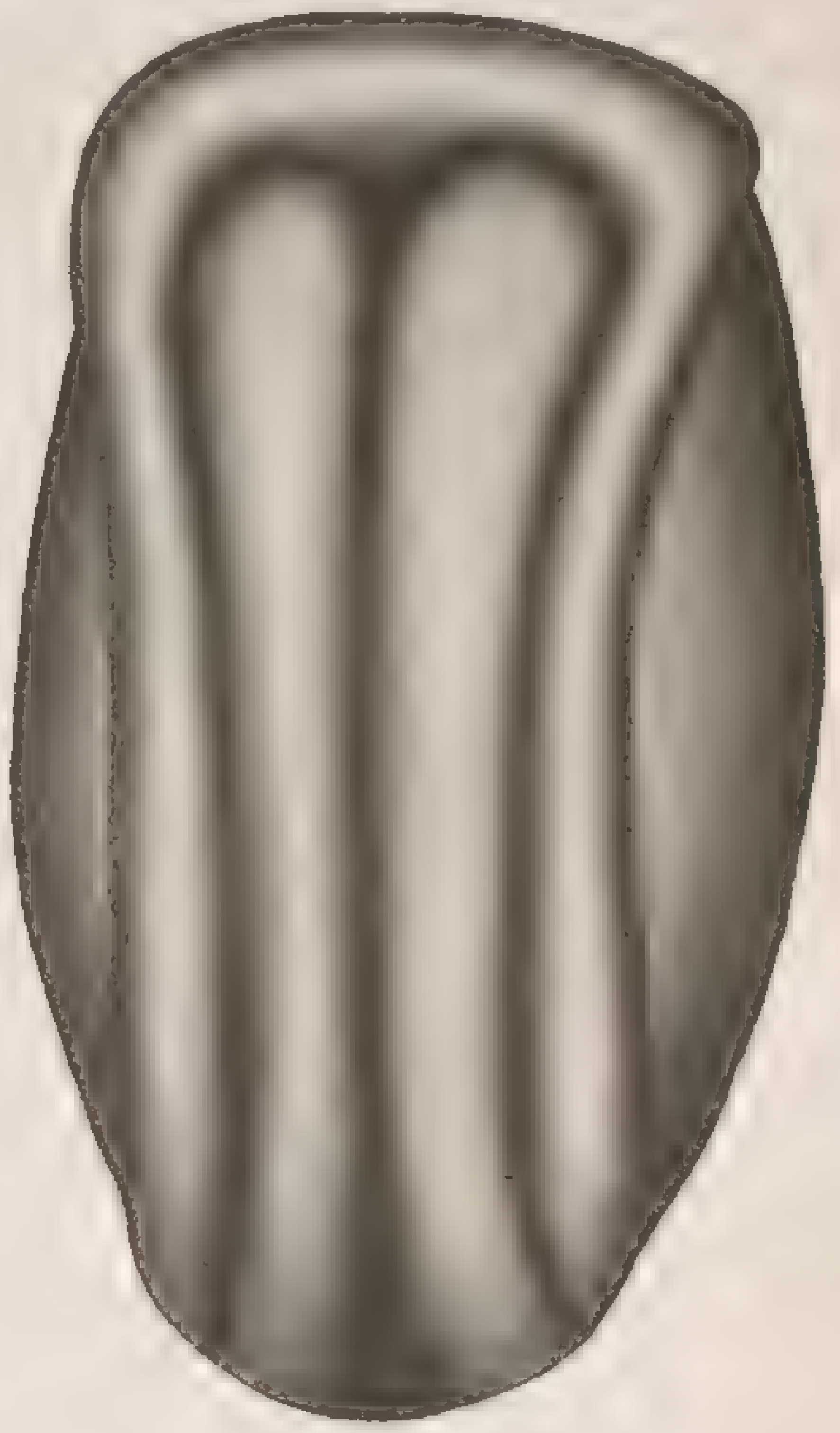

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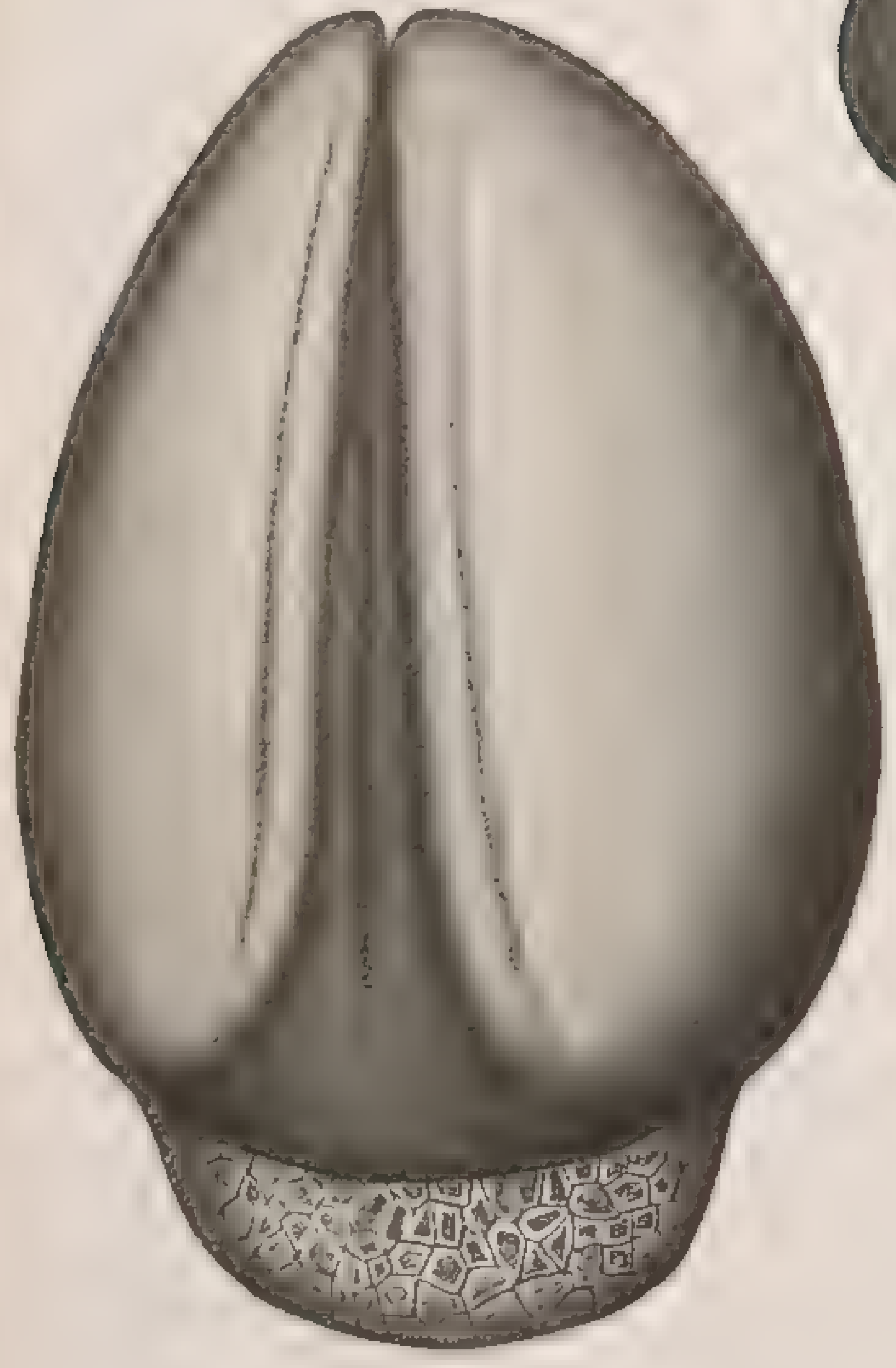

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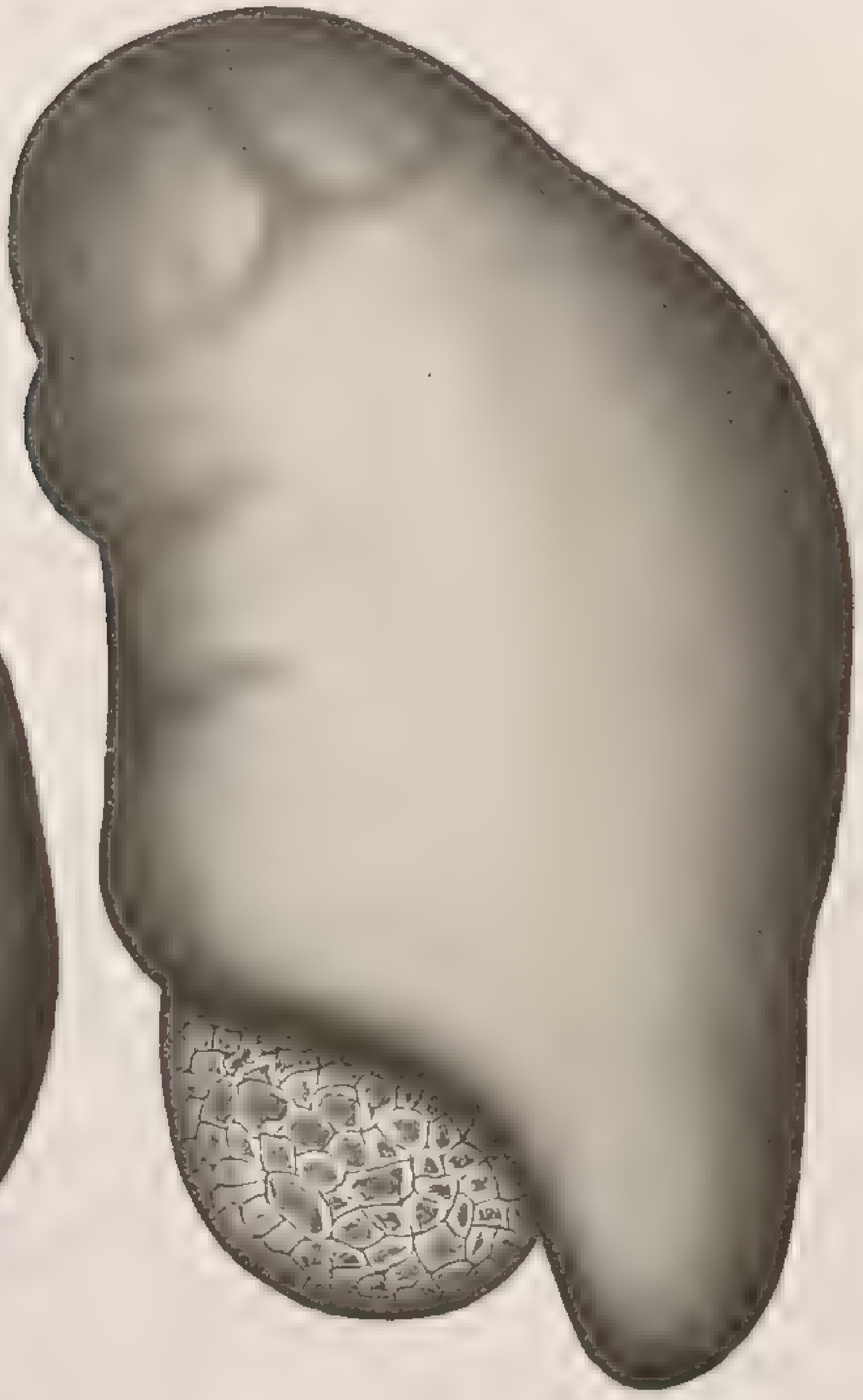

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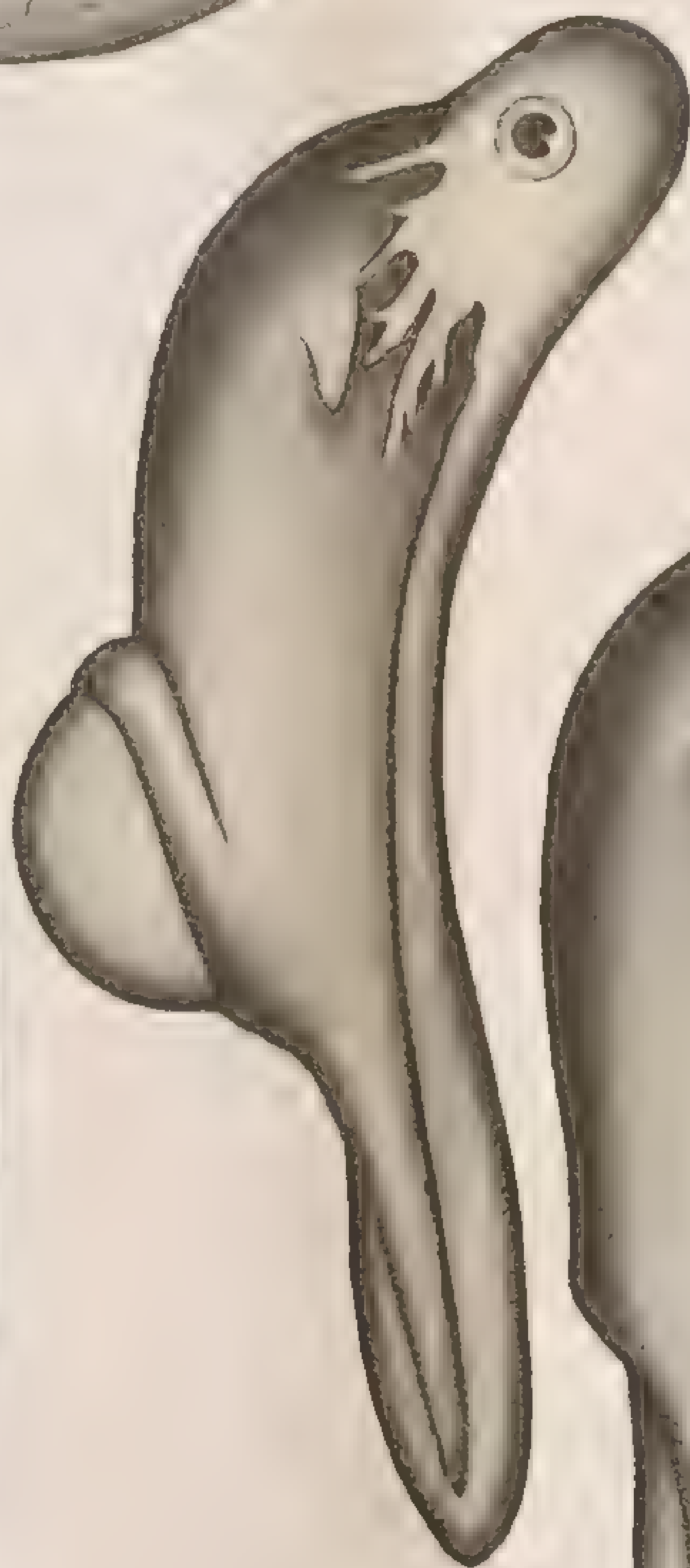

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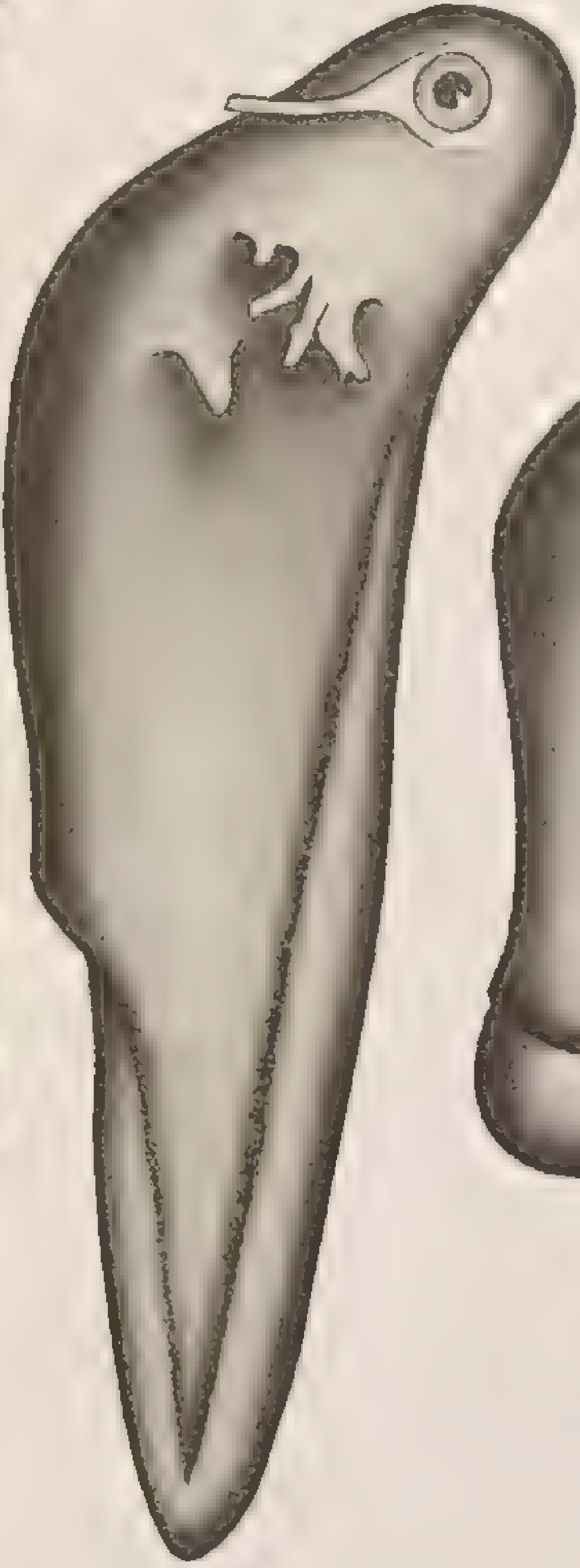

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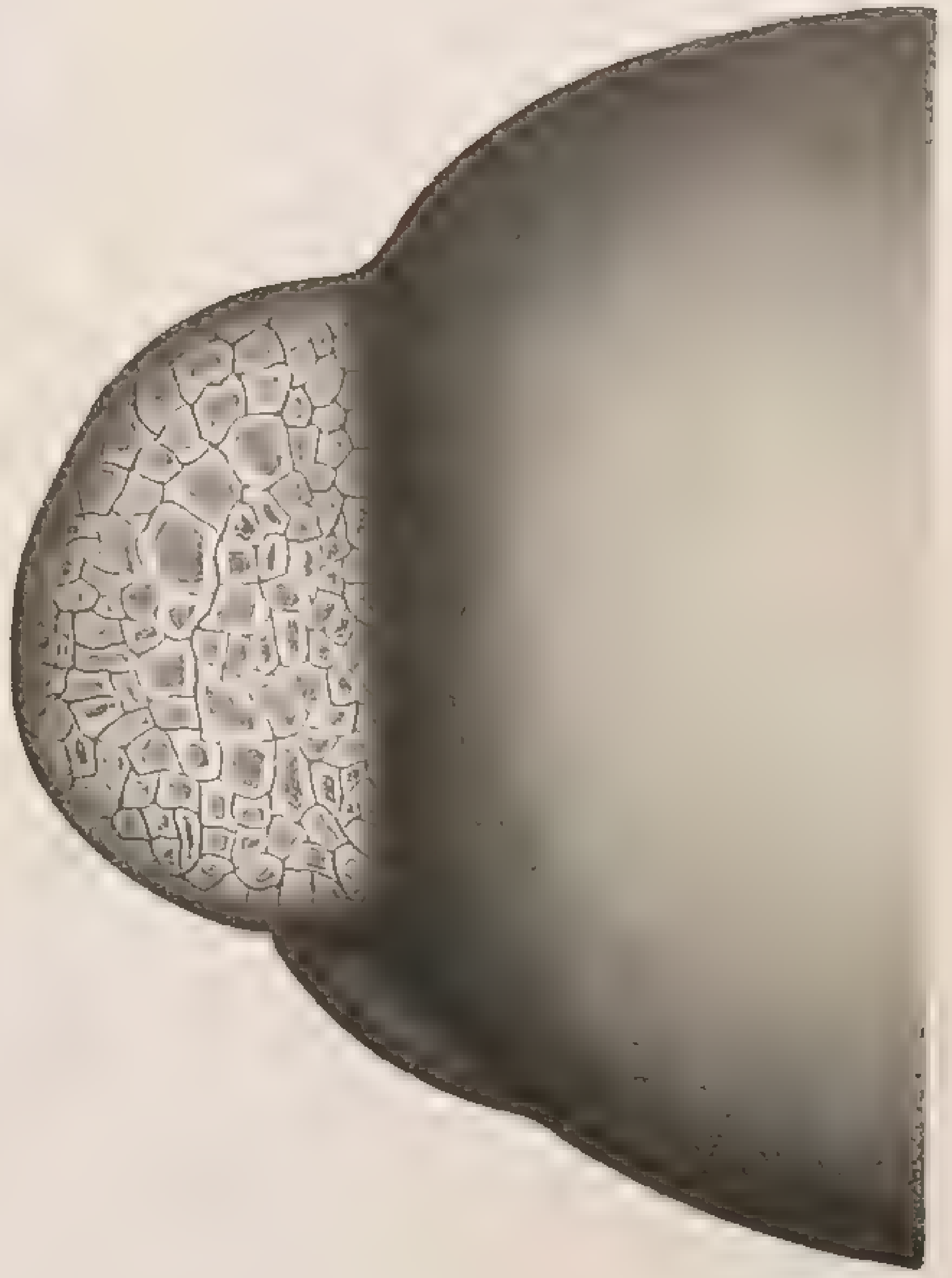

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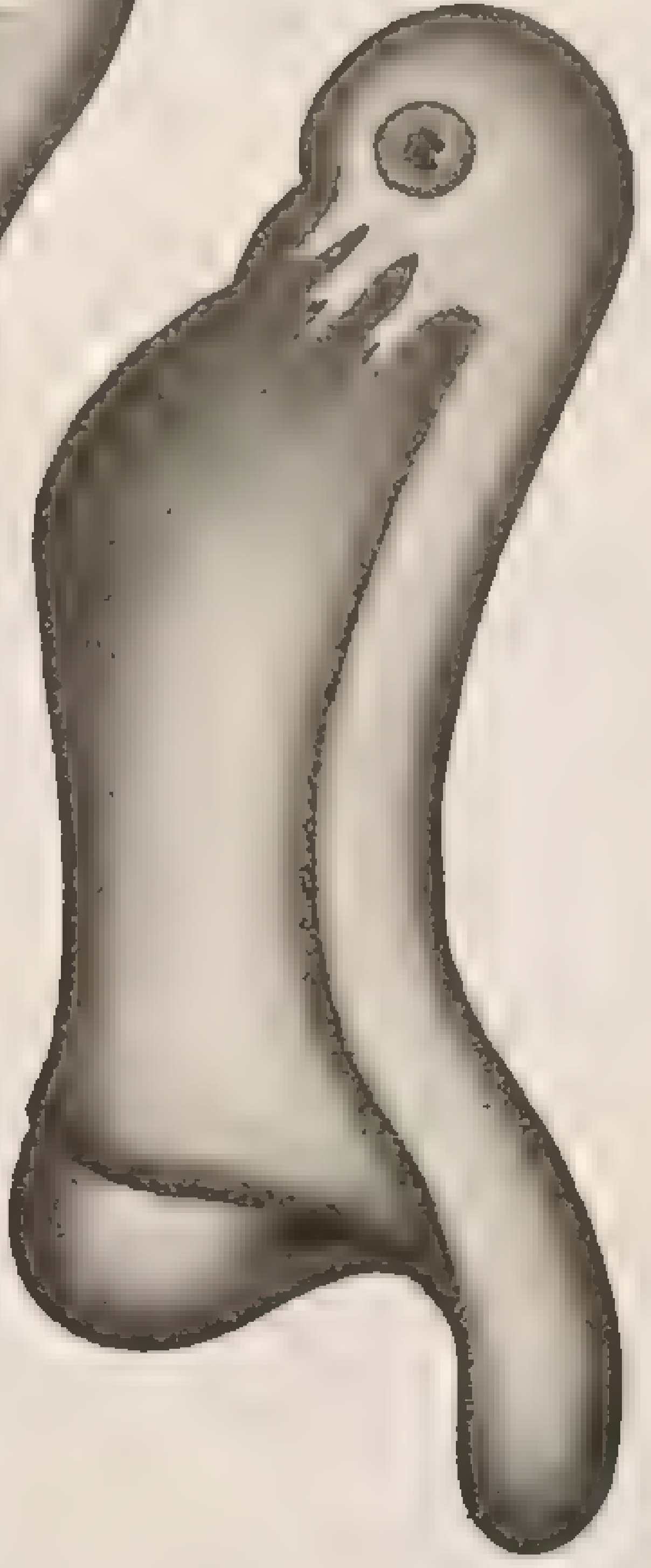

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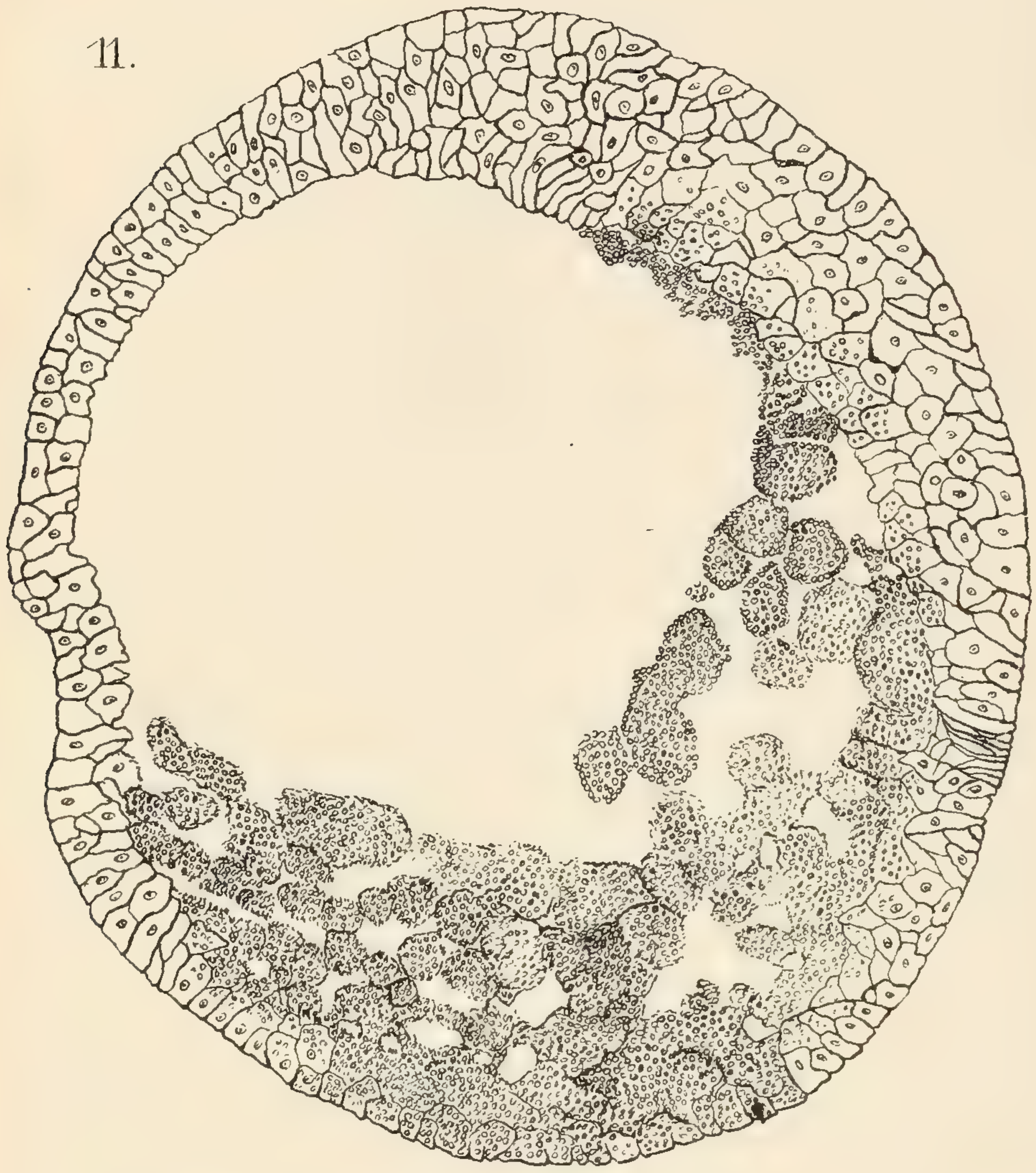

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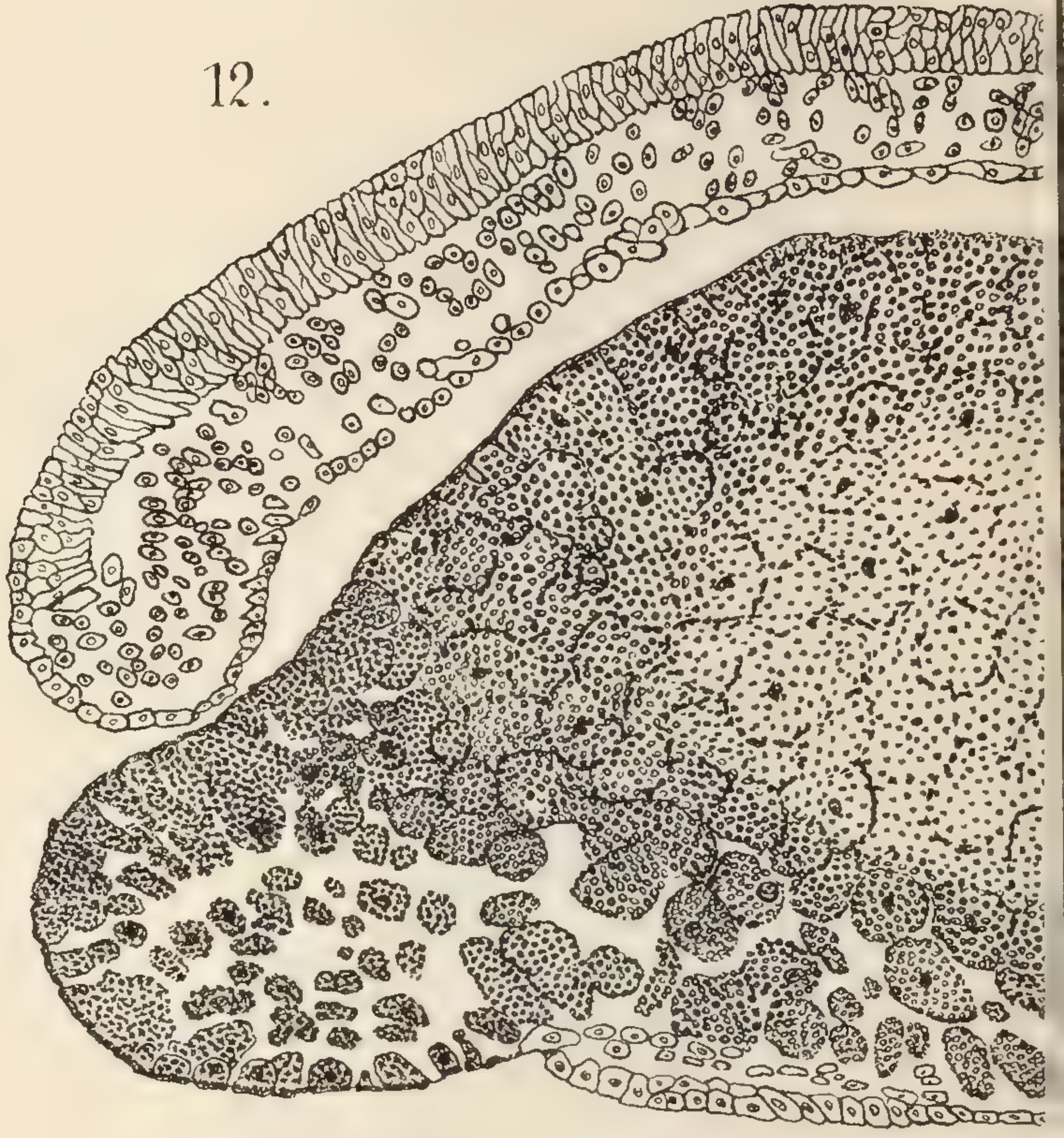

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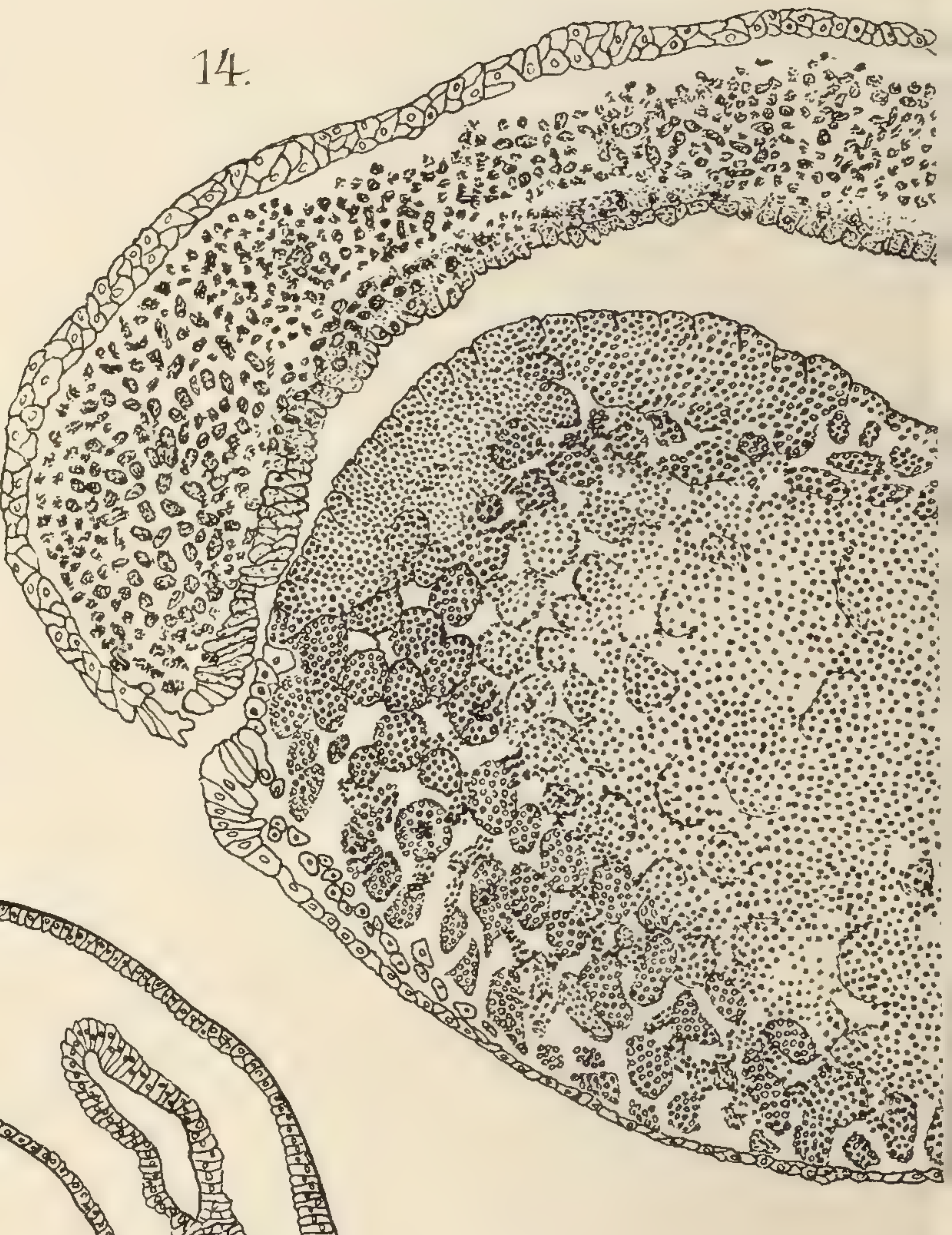

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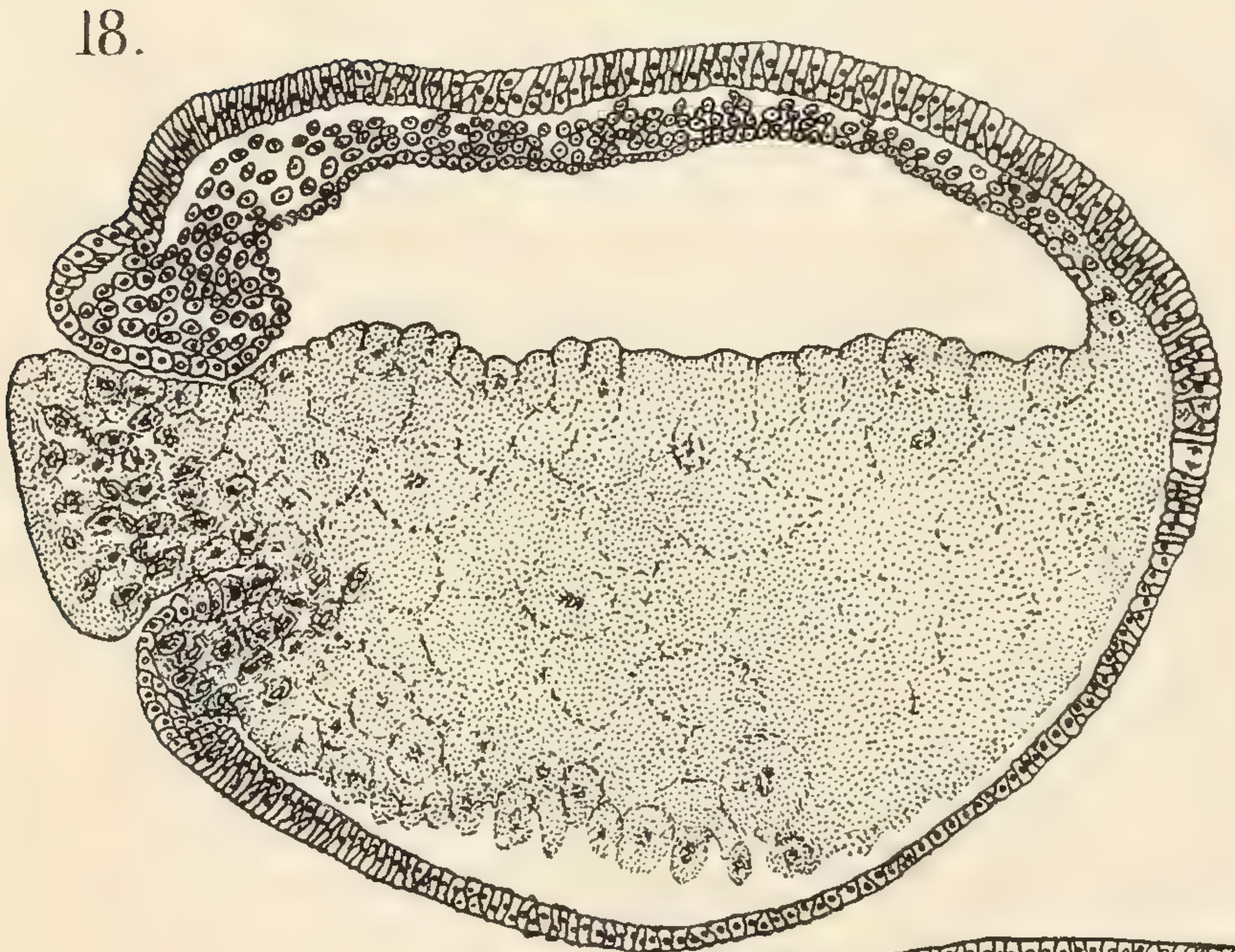

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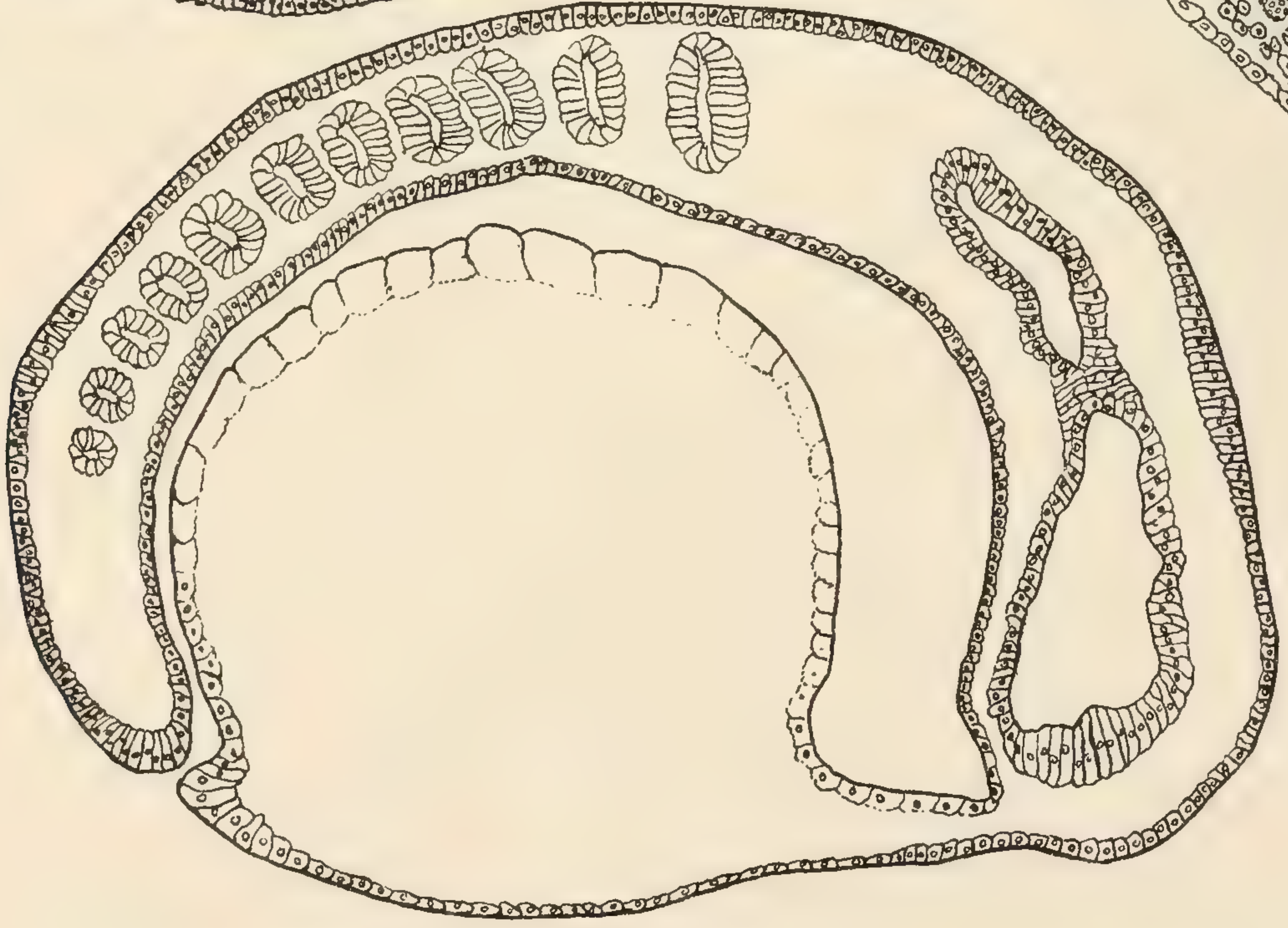

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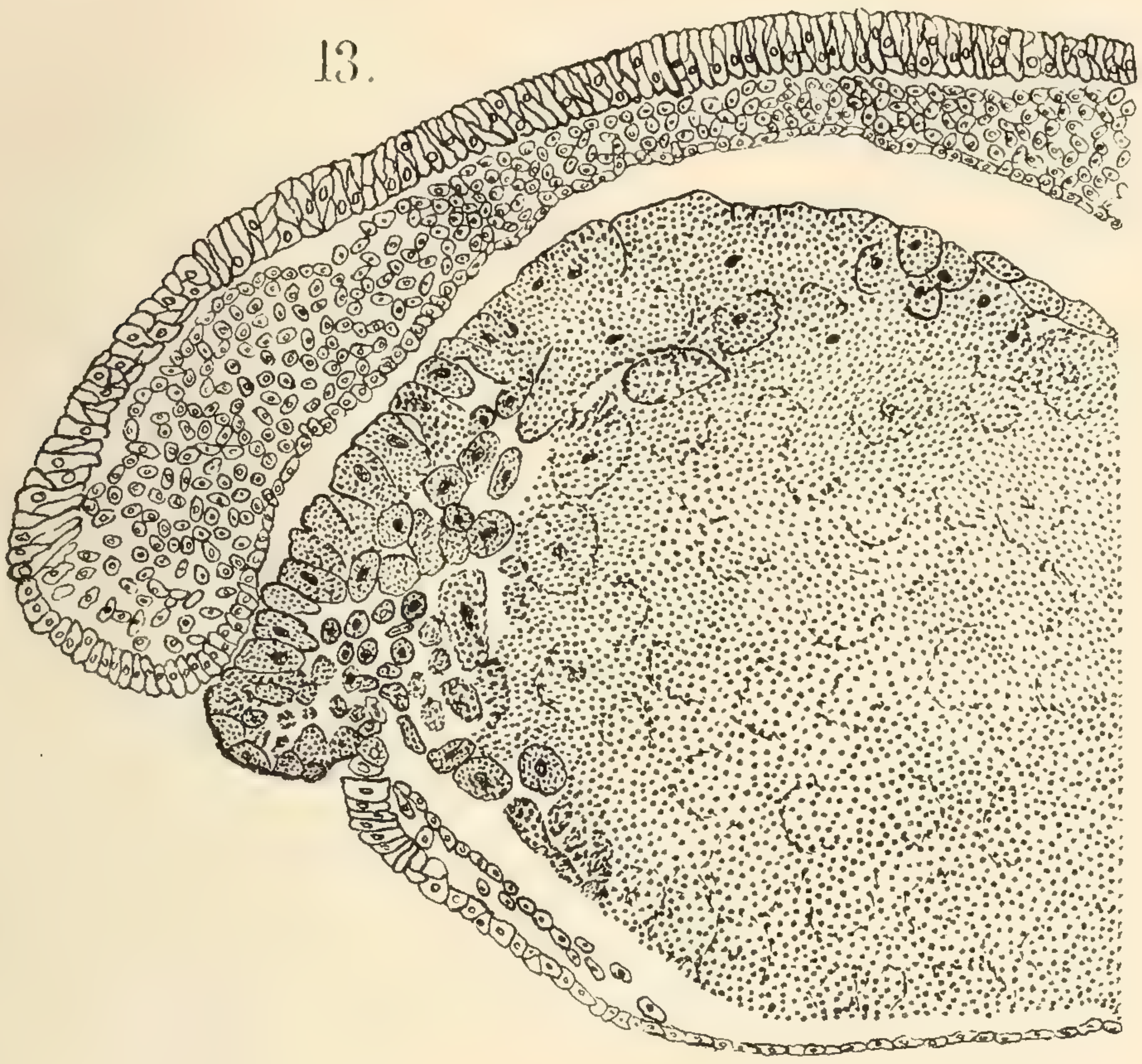

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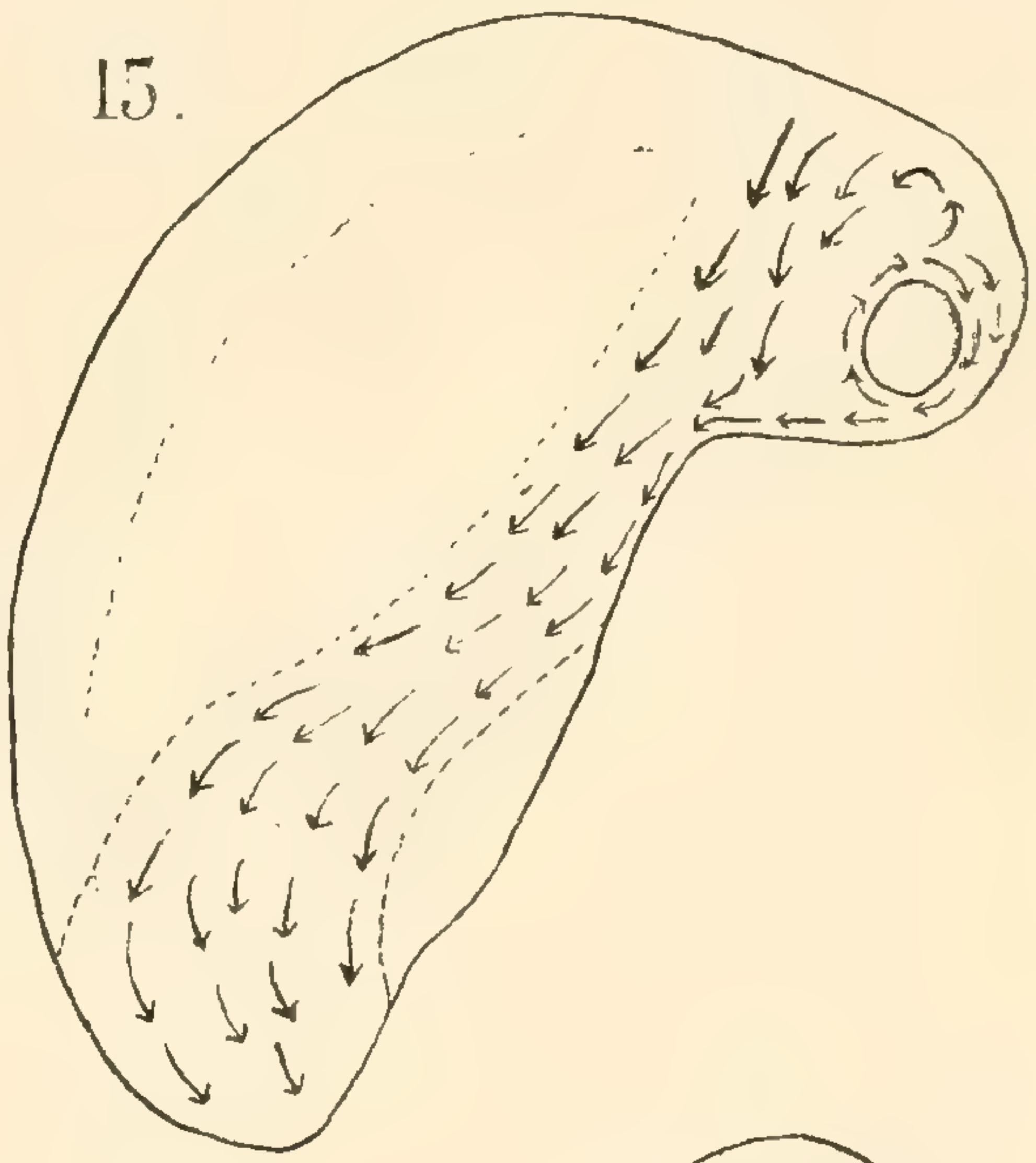

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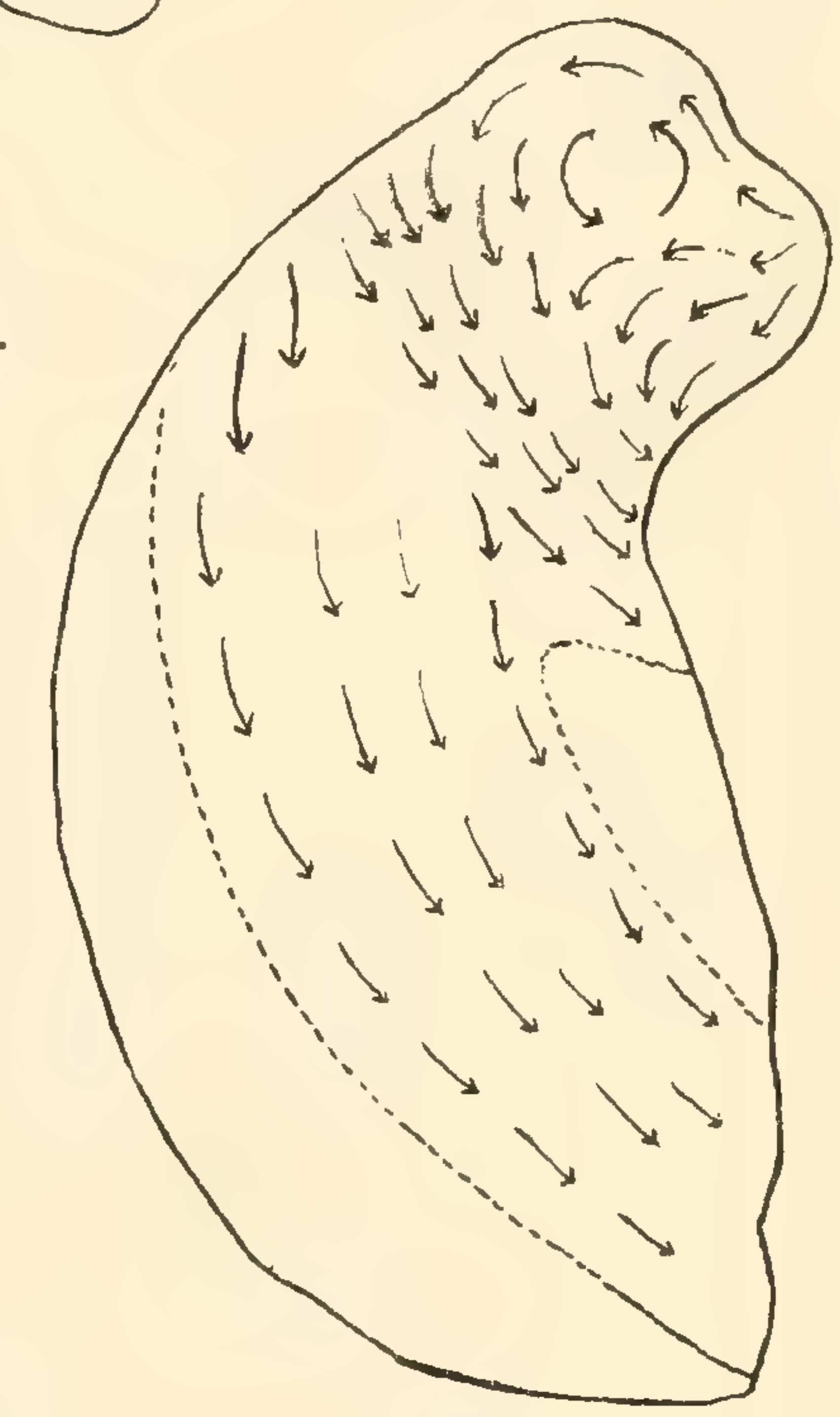

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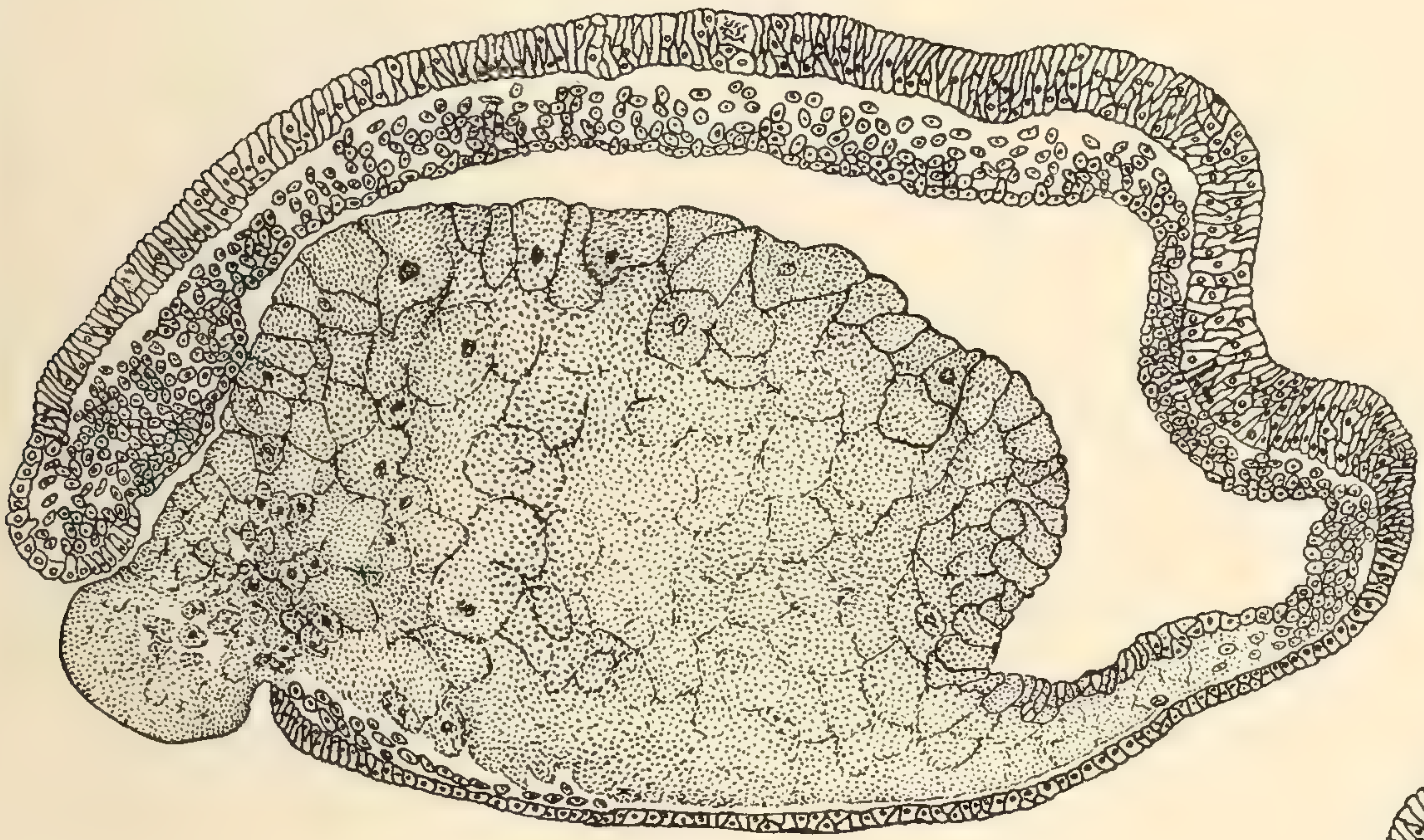

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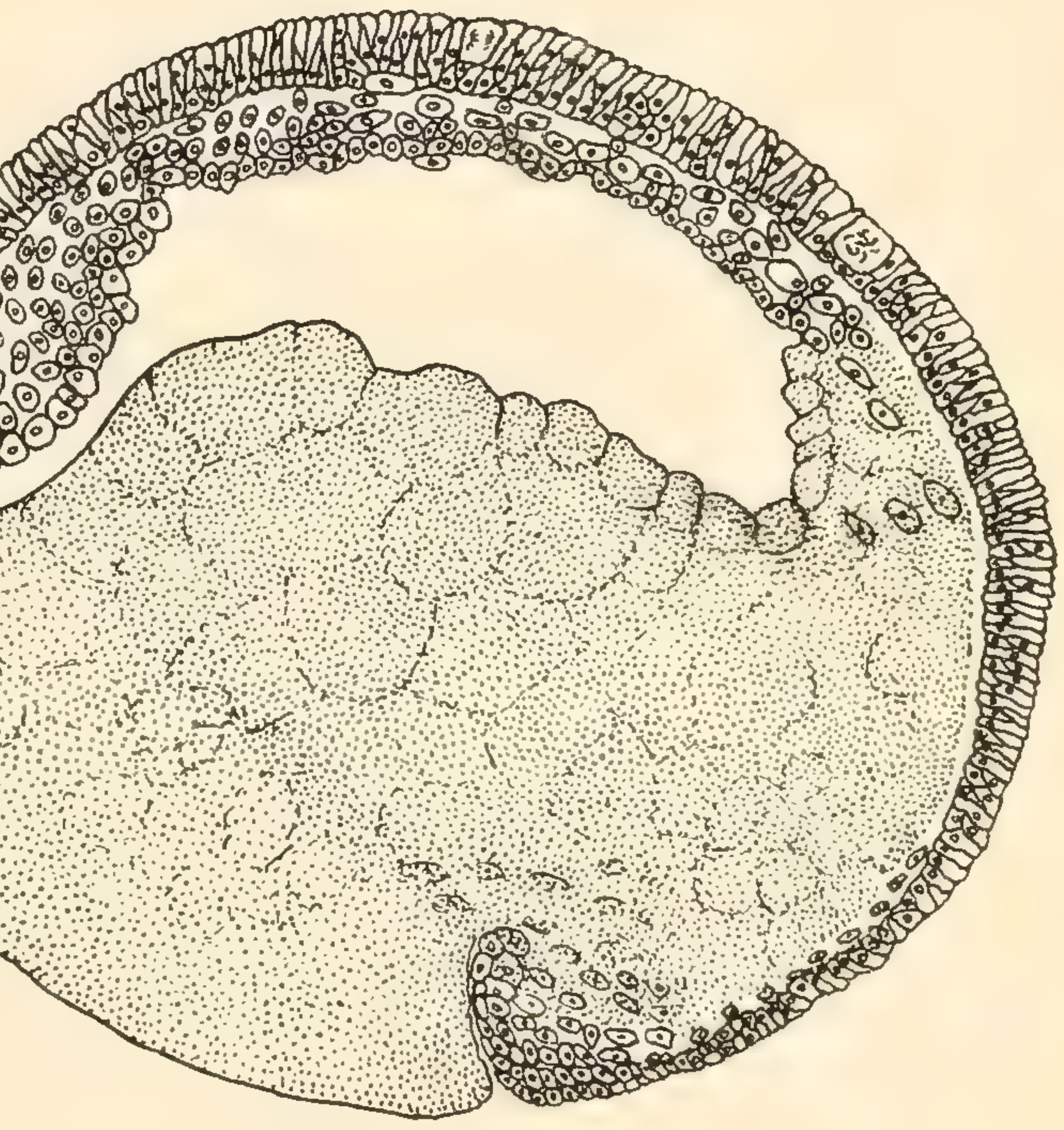

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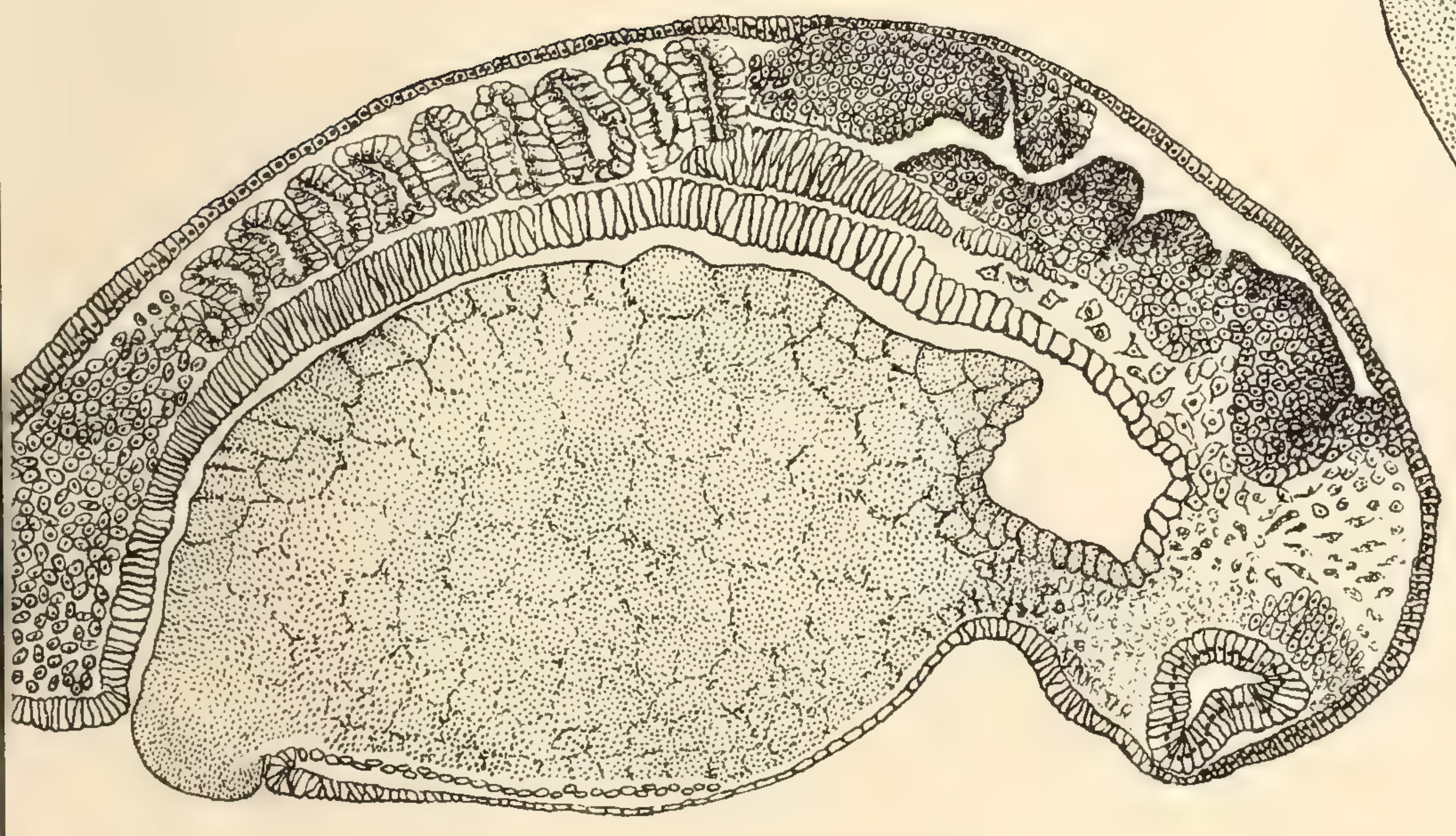

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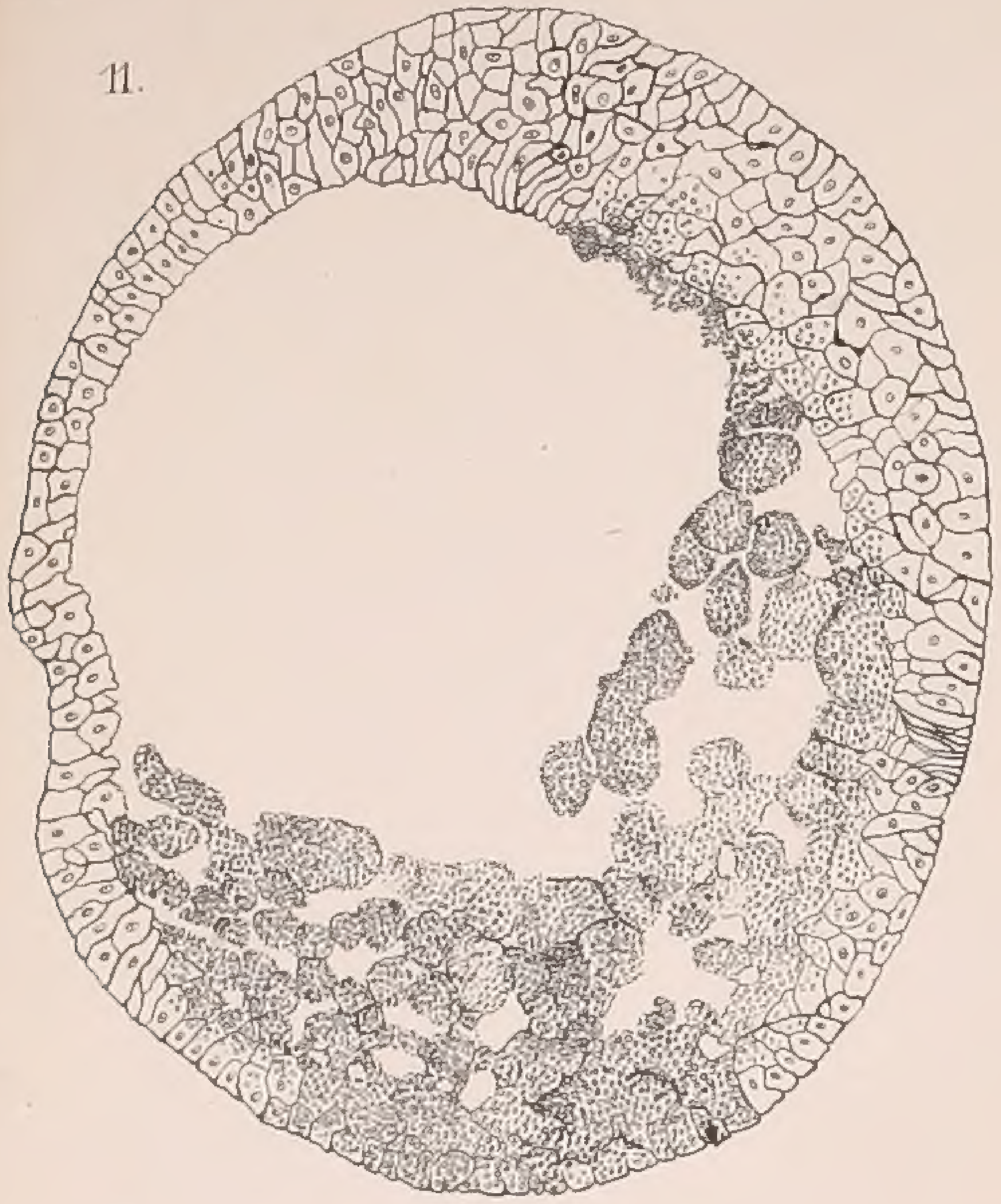

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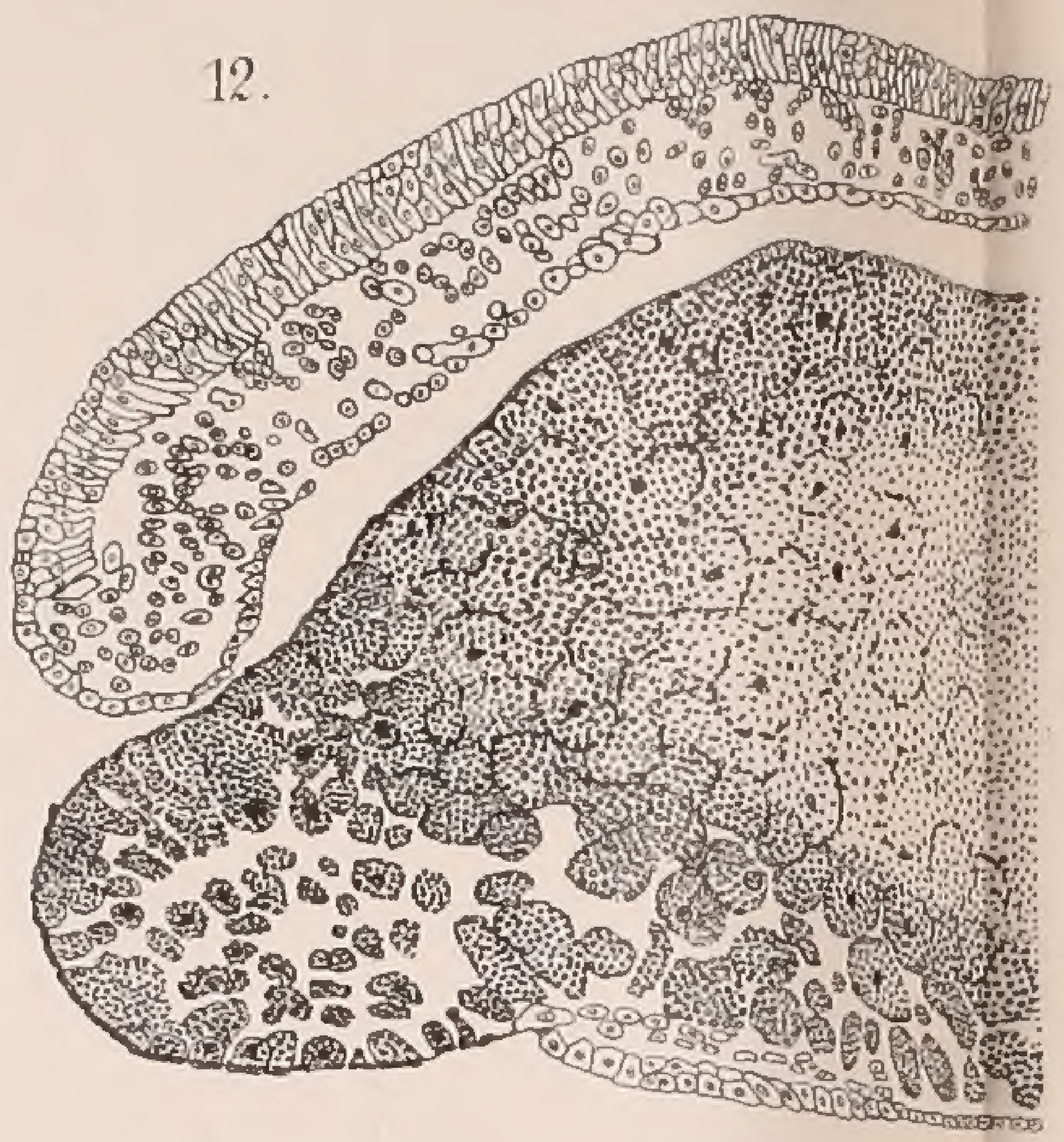

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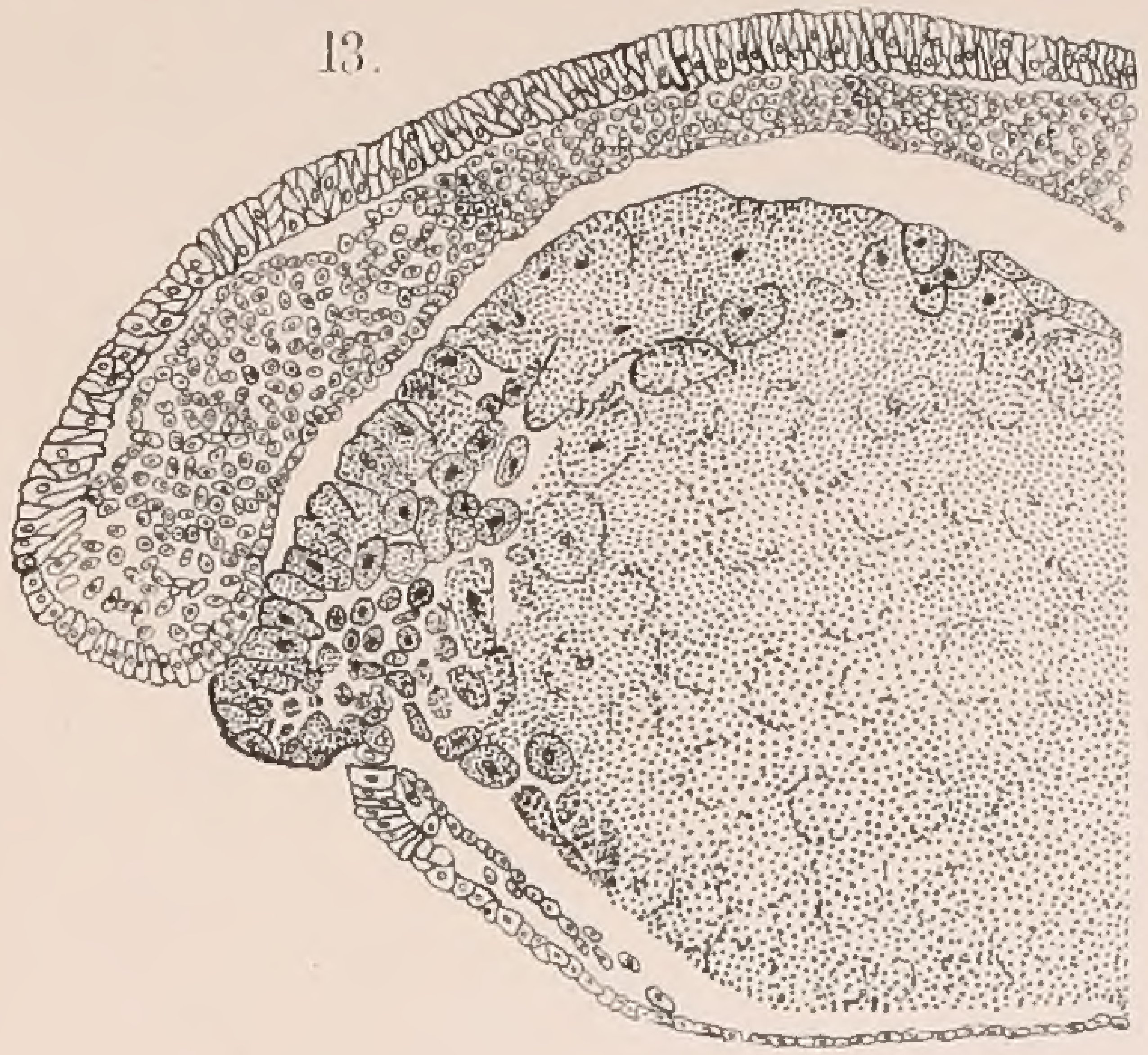

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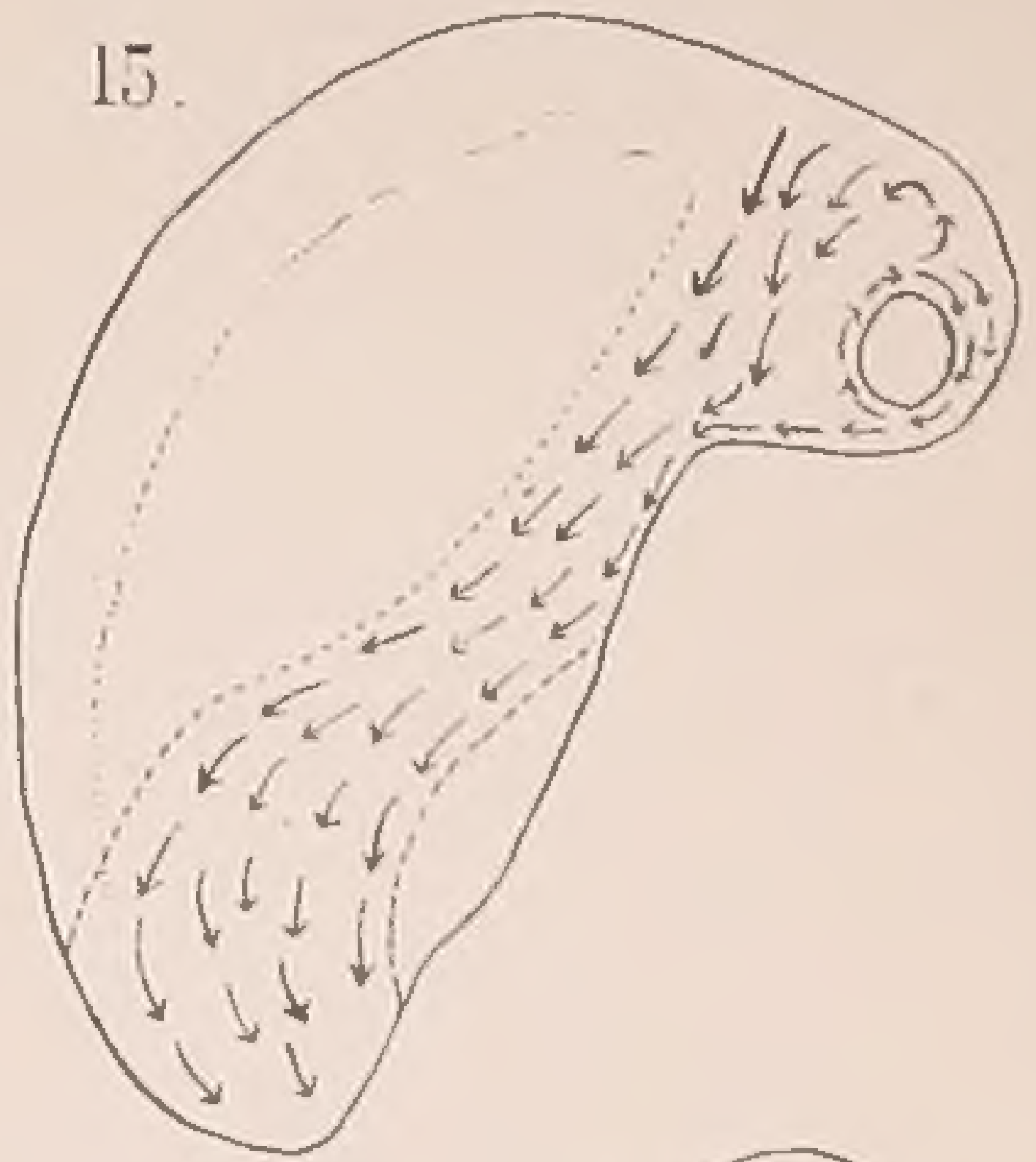

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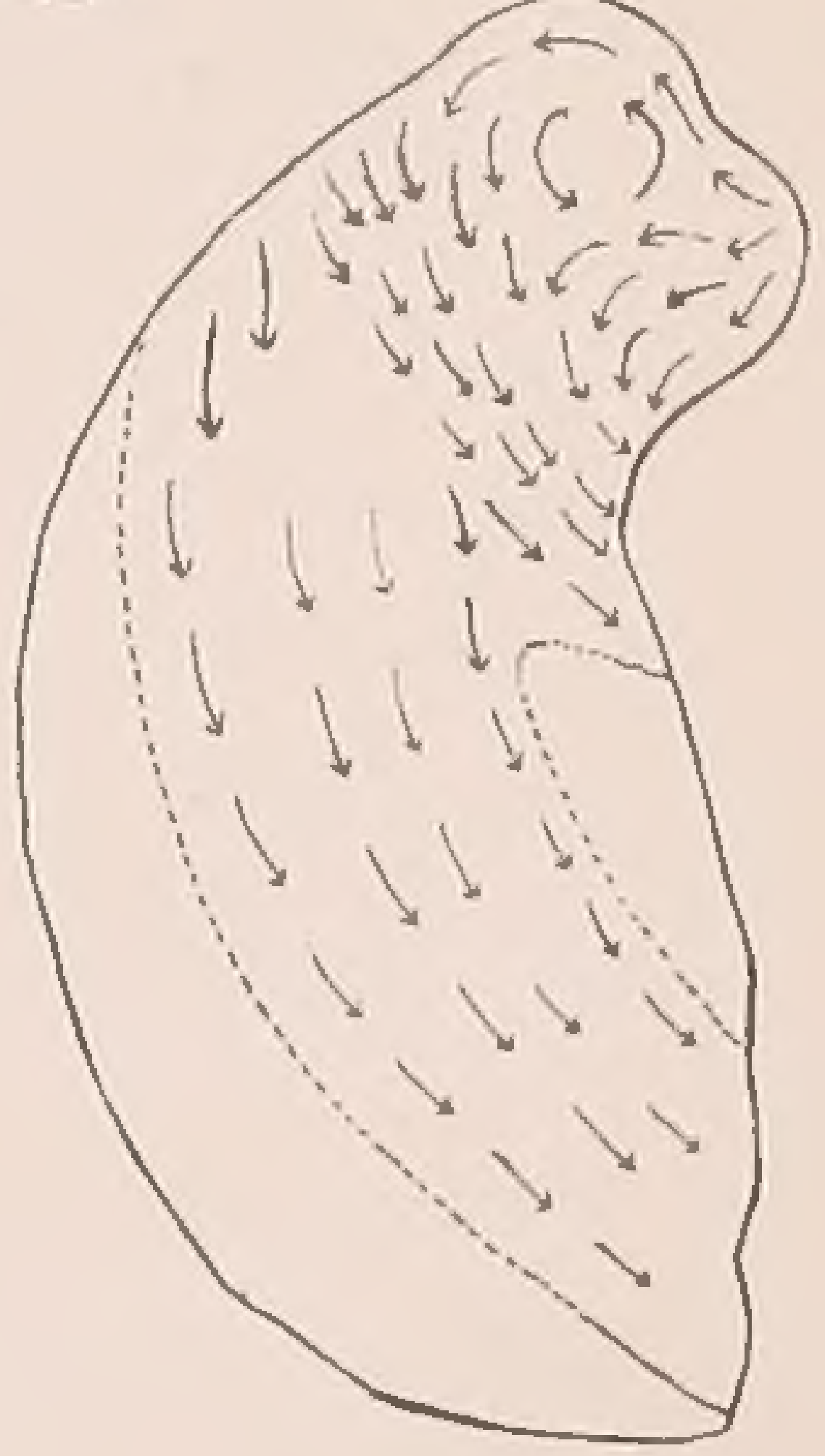

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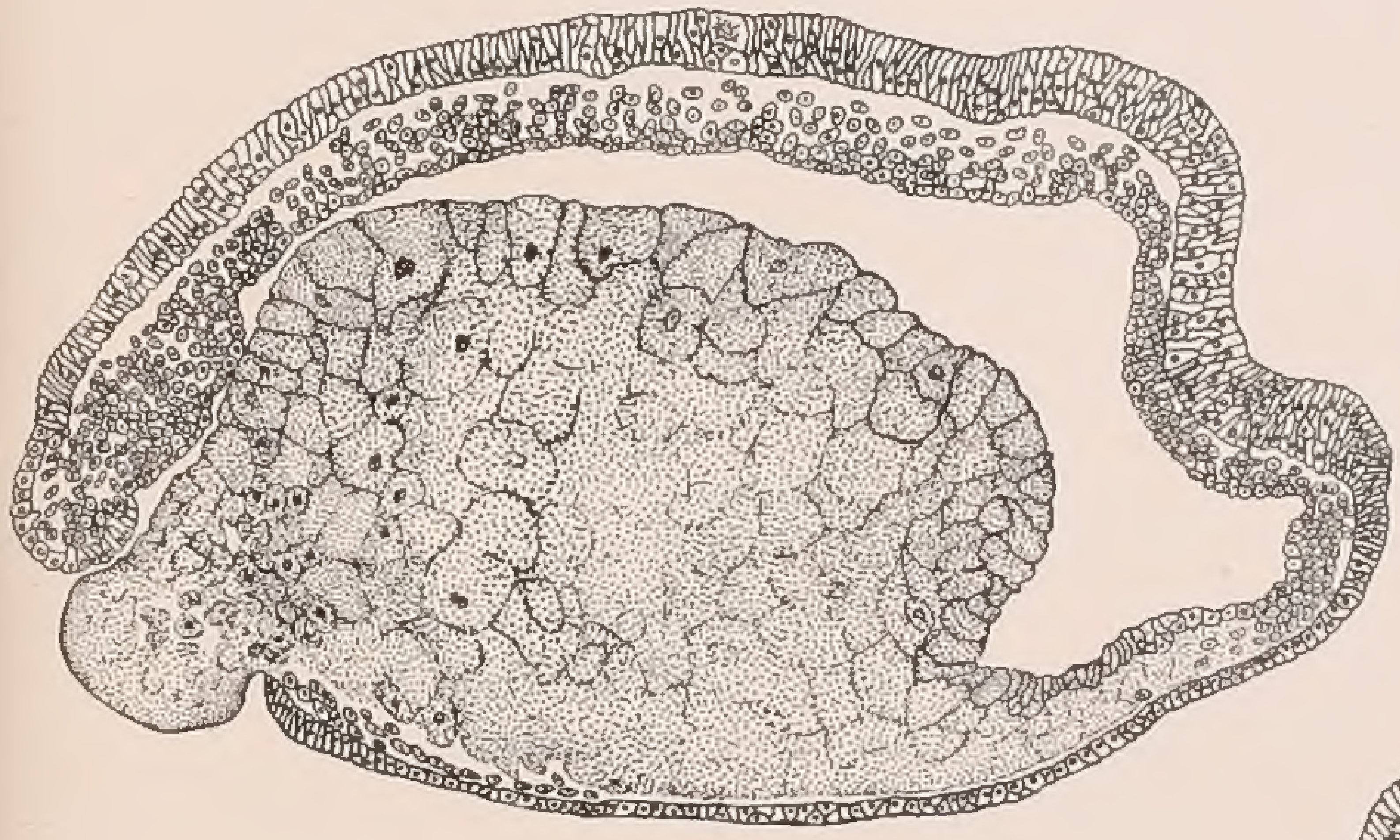

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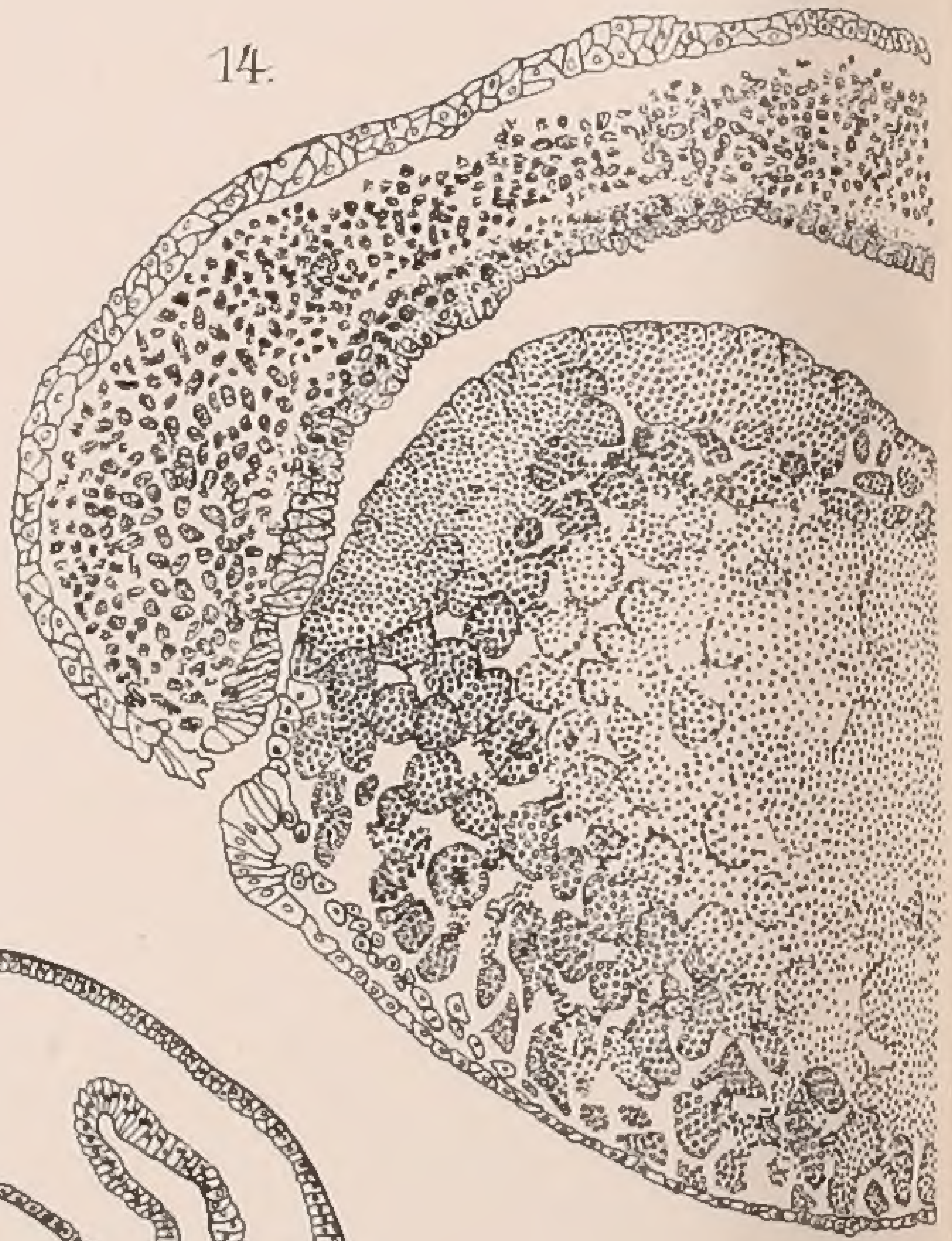

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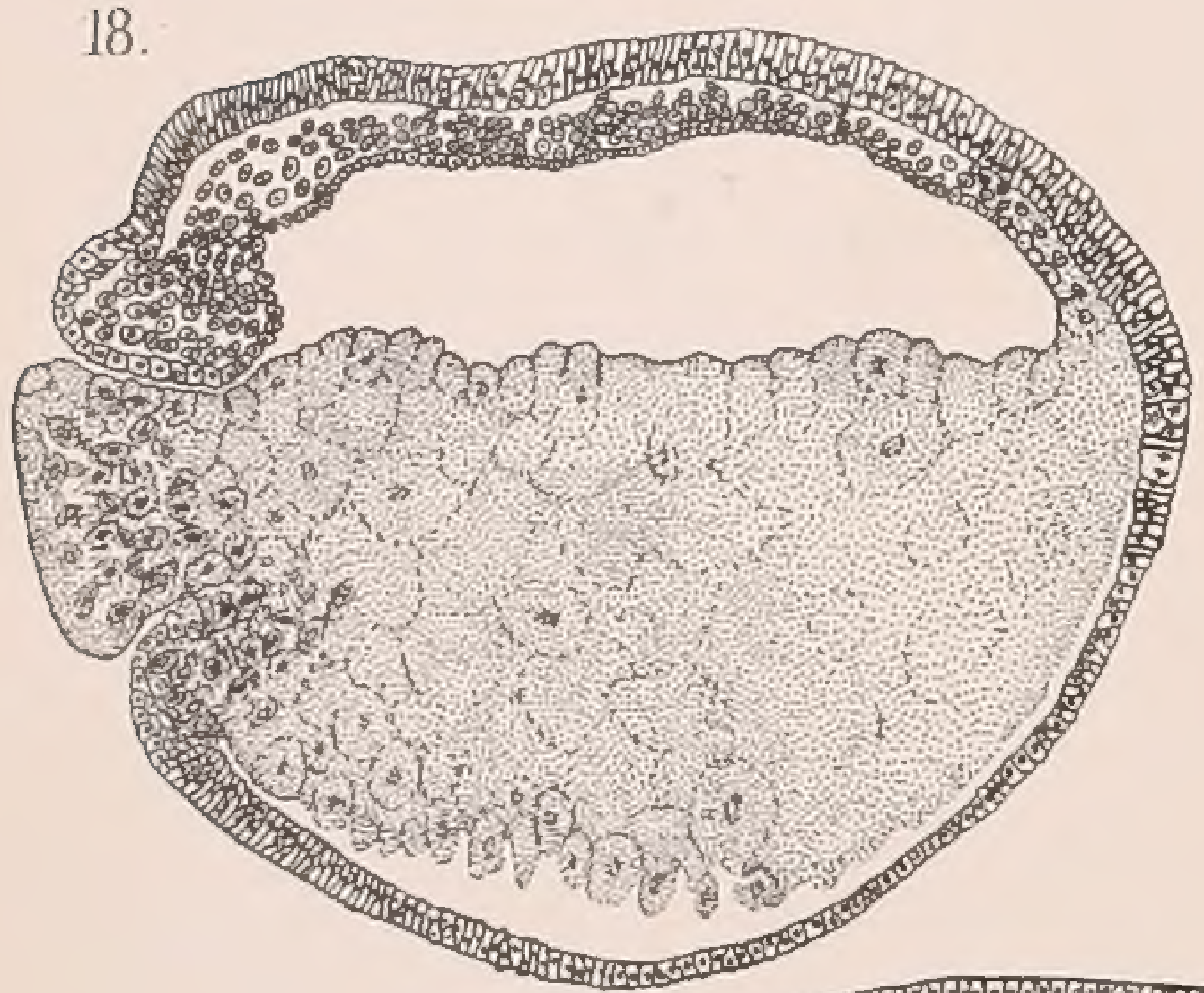

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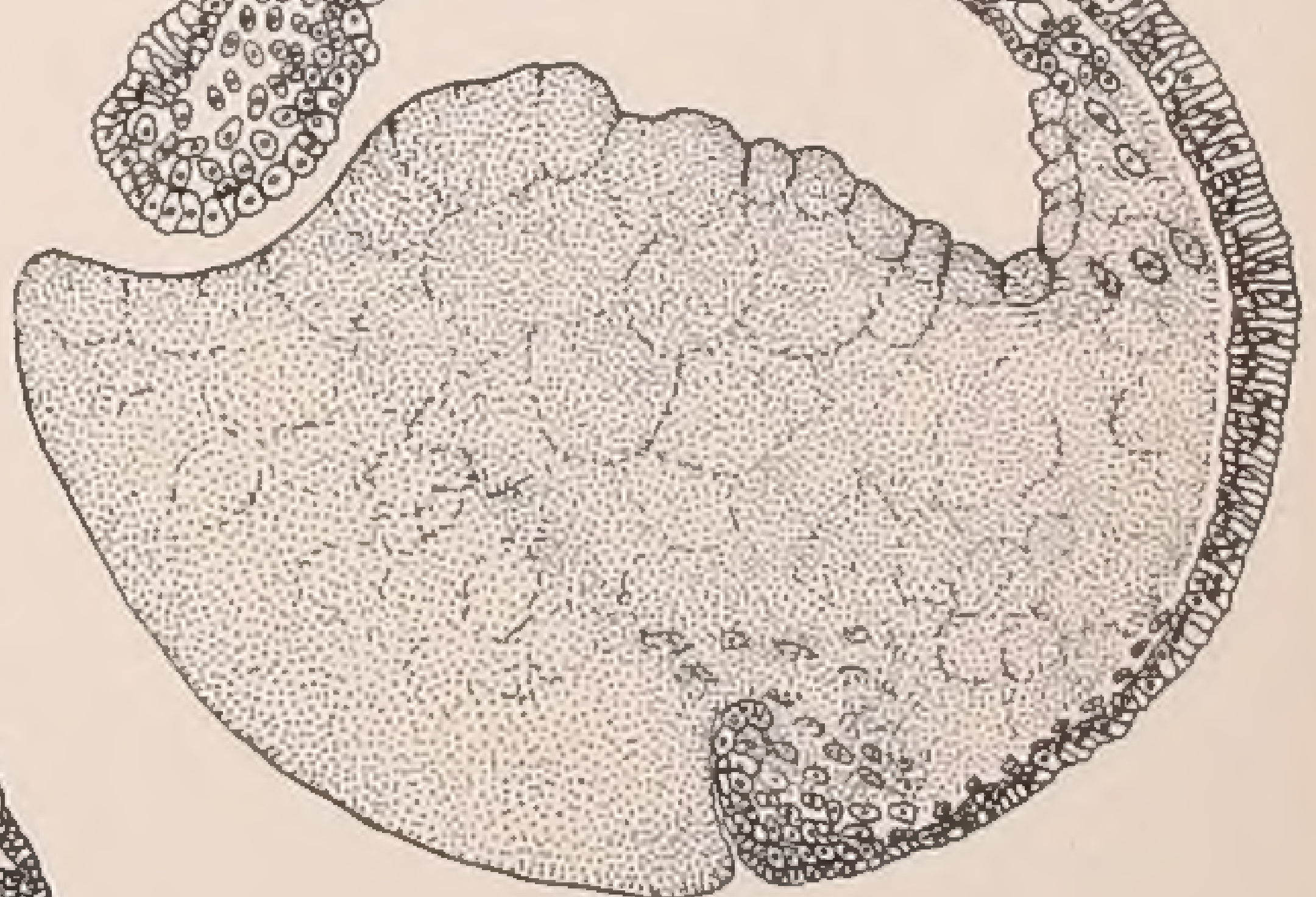

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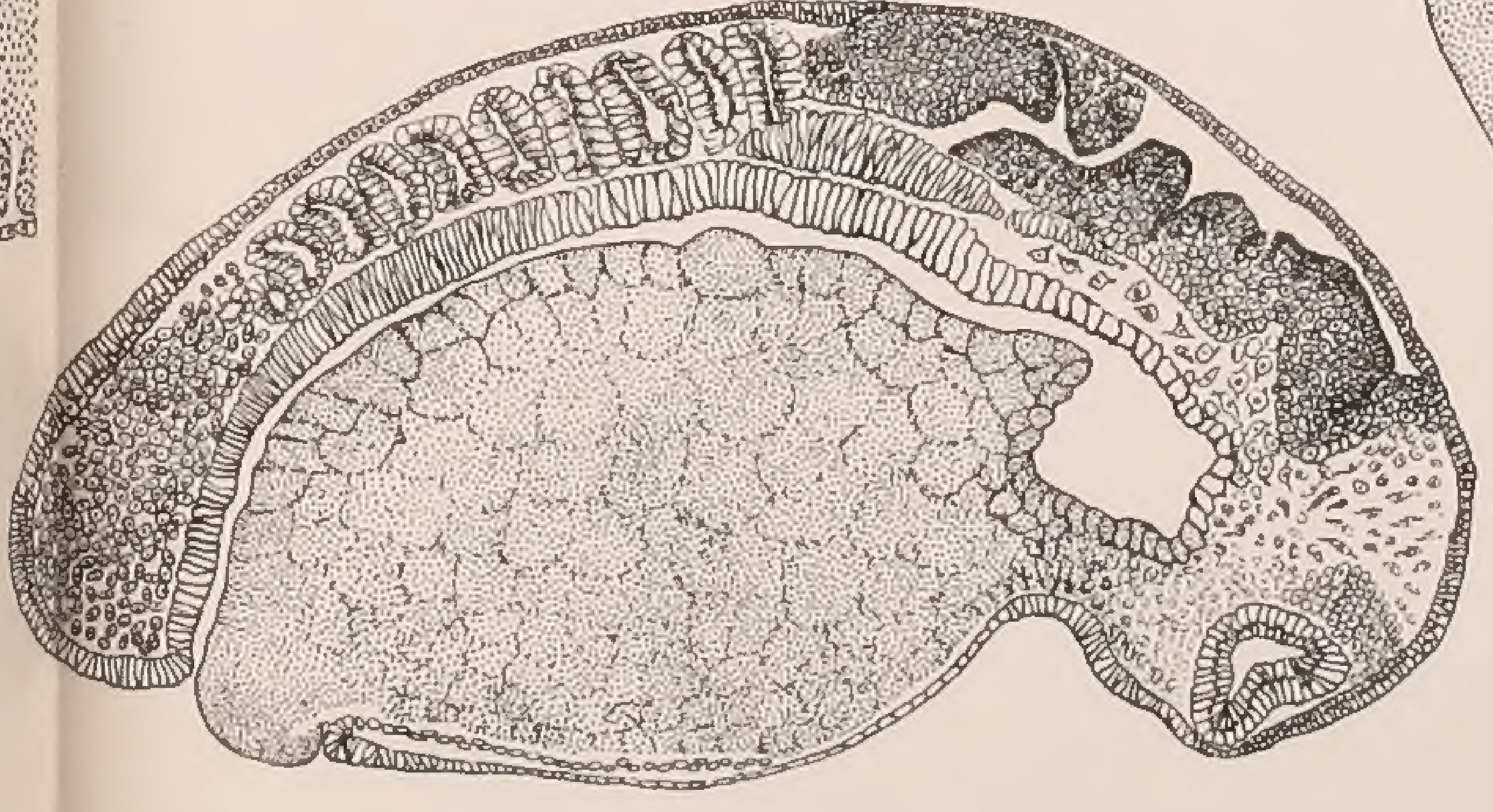

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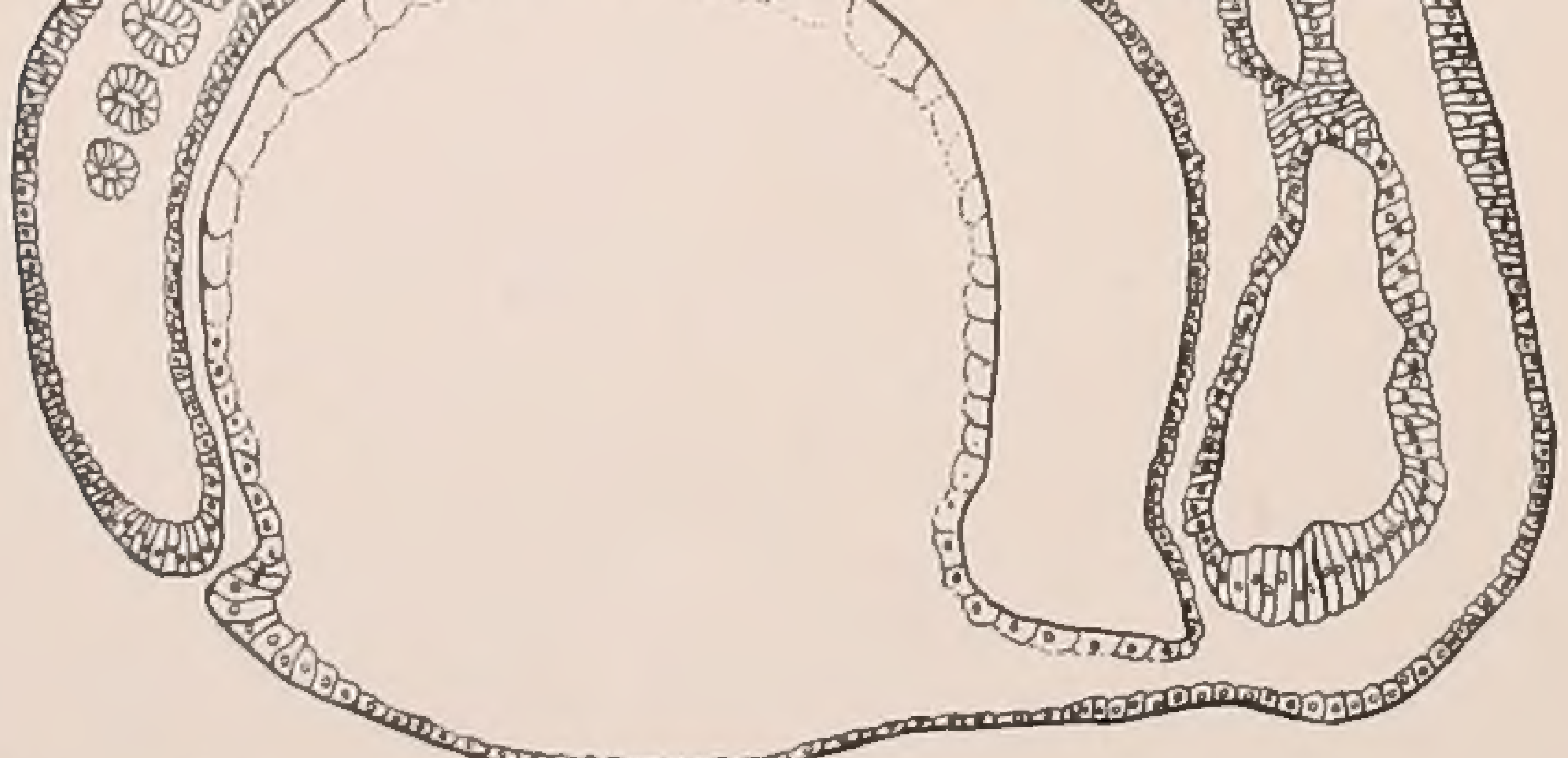

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